



## **Are Free Ion Activity Models Sufficient Alternatives to Biotic Ligand Models in Evaluating Metal Toxic Impacts in Terrestrial Environments?**

**Owsianiak, Mikolaj; Rosenbaum, Ralph K.; Larsen, Henrik Fred; Hauschild, Michael Zwicky**

*Publication date:*  
2011

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*

Owsianiak, M., Rosenbaum, R. K., Larsen, H. F., & Hauschild, M. Z. (2011). *Are Free Ion Activity Models Sufficient Alternatives to Biotic Ligand Models in Evaluating Metal Toxic Impacts in Terrestrial Environments?*. Poster session presented at Society of Environmental Toxicology and Chemistry : Navigating Environmental Challenges: Historical Lessons Guiding Future Directions, Boston, Massachusetts, United States.

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# abstract book



## **Society of Environmental Toxicology and Chemistry North America 32<sup>nd</sup> Annual Meeting**

Navigating Environmental Challenges:  
Historical Lessons Guiding Future Directions

Hynes Convention Center | Boston, Massachusetts  
13–17 November 2011



# Abstract Book

SETAC North America 32<sup>nd</sup> Annual Meeting

## Table of Contents

### Platform Abstracts

Monday.....	5
Tuesday.....	48
Wednesday.....	91
Thursday.....	132

### Poster Abstracts

Monday (MP).....	175
Tuesday (TP).....	227
Wednesday (WP).....	286
Thursday (RP).....	347

### Indices

Author Index.....	408
Affiliation Index.....	424
Session Index.....	431
Keyword Index.....	433

This book comprises the abstracts of the presentations for the platform and poster sessions of the 32<sup>nd</sup> Annual Meeting in North America of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the John B. Hynes Veterans Memorial Convention Center in Boston, Massachusetts, 13–17 November 2011. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order. In each abstract, the presenting author's name is underlined.

The author index cross-references the corresponding abstract numbers. Session and keyword indexes are also included.

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International Standard Serial Number 1087-8939

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## A Professional Society for Environmental Scientists and Engineers and Related Disciplines Concerned with Environmental Quality

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a nonprofit, professional society established to provide a forum for individuals and institutions engaged in the study of environmental problems, management and regulation of natural resources, education, research and development, and manufacturing and distribution.

Specific goals of the society are:

- Promote research, education, and training in the environmental sciences
- Promote the systematic application of all relevant scientific disciplines to the evaluation of chemical hazards
- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia, and other segments of society involved in the use, protection, and management of our environment

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- Provide funds for education and training through the SETAC Scholarship/Fellowship Program
- Organize and sponsor chapters and branches to provide a forum for the presentation of scientific data and for the interchange and study of information about local and regional concerns
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**229 South Baylen Street, 2<sup>nd</sup> Floor**  
**Pensacola, Florida, 32502 USA**  
**T +1 850 469 1500**  
**F +1 850 469 9778**  
**E [setac@setac.org](mailto:setac@setac.org)**

**Avenue de la Toison d'Or 67**  
**B-1060 Brussels, Belgium**  
**T +32 2 772 72 81**  
**F +32 2 770 53 86**  
**E [setaceu@setac.org](mailto:setaceu@setac.org)**

**[www.setac.org](http://www.setac.org)**  
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**1 Environmental Radiation: Comparing Risk Thresholds and Improving Risk Assessment** M.E. Bates, US Army Corps of Engineers, Environmental Lab, Engineer Research and Development Center; L.J. Valverde, US Army Corps of Engineers, Headquarters; J.T. Vogel, US Army Corps of Engineers, Environmental Lab, Engineer Research and Development Center; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center. In the months following the March 2011 nuclear disaster at the Japanese Fukushima I nuclear power plant, the eyes of the public, worldwide, turned a new scrutiny towards nuclear policy. In the United States, this scrutiny often focuses on the US Nuclear Regulatory Commission (NRC) and questions whether current regulations adequately protect the public from risks of nuclear harm. The question of appropriate risk protection can be addressed in two parts, asking whether current regulatory risk thresholds are reasonable and asking whether the calculated risks being compared to the thresholds accurately capture the risk inherent in the system. To address the first question, NRC risk benchmarks are compared with those of other public agencies. A comparison with agencies not under particular public scrutiny finds NRC risk thresholds among the most conservative of the assessed agencies, suggesting that NRC's current regulatory framework is consistent with broader social ideals. Learning from the lessons in Japan, the second question implies that, at least in the Fukushima scenario, the true risks of disaster were likely underestimated in the nuclear risk calculations. To improve nuclear risk assessment, the accident suggests that it is important to bring systemic reasoning to the risk management process and evaluate the resilience of the system to minimize the potential for surprise.

**2 Human Health Risks of Petroleum Coke as Fuel for Electric Power Generation** B. Magee, ARCADIS; C. Ryan, K. Gordon, A. Bielecki, New Brunswick Power Corporation. The New Brunswick Power Corporation sought regulatory approval for a performance project with a mixture of petroleum coke and heavy fuel oil (HFO) as fuel at their electric power generation station in Lorneville, New Brunswick. Petroleum coke is a less costly fuel than HFO, but the human health risks posed by handling of petroleum coke and emissions from the combustion of the petroleum coke/HFO mixture required detailed assessment. A comprehensive multipathway human health risk assessment was performed in accordance with United States Environmental Protection Agency (USEPA) combustor risk assessment guidance. The results of this risk assessment demonstrated that use of petroleum coke as fuel was acceptable, and the performance project was undertaken. Data from emissions testing performed during the combustion of the fuel mixture were subsequently used to update the risk assessment in support of environmental permits to allow use petroleum coke/HFO as a routine fuel. This presentation will present the results of the risk assessment showing that petroleum coke is an environmentally acceptable fuel. In addition, in response to worker concerns about the handling of petroleum coke, a comprehensive literature review was performed, and a toxicological dossier on petroleum coke was prepared. The presentation will summarize the major findings of the literature review.

**3 Incorporating Multi Criteria Decision Analysis in an Integrated Life Cycle Sustainability Assessment of Palm Oil Biodiesel** Y. Manik, Univ of Maine, Research Group of Industrial Ecology, LCA and System Sustainability (IELCASS); A. Halog, Univ of Maine, Research Group of Industrial Ecology, LCA and System Sustainability (IELCASS), Univ of Maine, Assistant Professor of Industrial Ecology and LCA. In the next few decades, worldwide production of palm oil demand is estimated to increase, particularly as an anticipation of the worldwide biodiesel demand. The massive growth of palm oil is causing enormous potential environmental problems, such as climate change, eco-toxicity, loss of biodiversity as well as societal issues like land conflicts and workers' rights. On the other hand, palm oil industry contributes significantly to the GDP and creates employment for millions of workers nationwide. In the pursuit of sustainable development, an integrated life cycle sustainability assessment framework is needed to enable to support policy and regulation formulation as well as for operational decision making. This study aims to demonstrate how various techniques, such as Life Cycle Assessment, Life Cycle Costing enhanced with Ecological Economics approach, and Social Impact Assessment are combined altogether to develop an integrated instruments that will be employed as a decision making tool within a Multi Criteria Decision Analysis of the integrated life cycle sustainability assessment of palm oil biodiesel, in which the multidimensionality of the sustainability goals and the complexity of socio-economic aspects are taken into account.

**4 Modeling Aeolian Transport of Contaminants for Long-Term Risk Assessment: Sensitivities to Succession, Disturbance and Future Climate** J.J. Whicker, Los Alamos National Laboratory, Waste and Environmental Services; T. Kirchner, New Mexico State Univ; B.D. David, J.P. Field, Univ of Arizona, School of Natural Resources and Dept of Ecology and Evolutionary Biology. Aeolian processes dominant redistribution of contaminated soil from protected, nonpublic areas to non-protected, public areas in semiarid sites. Wind transport rates are controlled by vegetation cover, yet long-term changes in vegetation associated with cycles of disturbance and succession is ignored by current sediment transport models. An empirically-based wind transport model (VMTran-Vegetation Moderated Transport) was developed to predict contaminant transport over 1000-year regulatory periods. VMTran simulates transport considering vegetation succession and ecosystem disturbances of three types (surface fire, crown fire, and drought-induced plant mortality) using disturbance rates for current and projected future climate. Simulation results for a Dept of Energy landfill show about 20% of surface contamination was transported offsite in 1000 years without considering disturbances whereas disturbances raised the amount eroded to about 80% of original concentration. More than 90% was eroded offsite under predicted future climate changes. Thus, consideration of vegetation succession and ecosystem disturbance is critical when evaluating public risk for long-term stewardship.

**5 Application of Decision-analytic Methods to Evaluate the Practicability of Green Roofs on DOD Buildings** D. Wang, Carnegie Mellon Univ; A.C. Bourne, US Army Corps of Engineers, New England District; Z.A. Collier, I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center. In the effort to comply with current sustainability initiatives, like the Army's net zero strategy for energy, water, and waste, Department of Defense (DOD) installations are faced with numerous options to meet environmental goals while continually upholding their missions. Among other innovative technologies, green roofs have the potential to be effective in reducing energy consumption, reducing storm water runoff, and improving water quality in addition to a host of other environmental benefits. Since green roof technologies are relatively new, there isn't extensive documentation on how to determine if installing a green roof is the right choice for specific installation needs, or how to make tradeoffs between various roof design parameters. In order to determine the practicability of green roof installation on new and existing DOD buildings, an integrated decision framework is proposed. This framework assesses the need for green roof installation, and the scope and engineering design of such projects from the standpoint of cost-effectiveness, landscape position, potential energy and water savings, and other sustainability efforts already in place. It uses multi-criteria decision analysis (MCDA) to compare green roofs with other innovative technologies, as well as to evaluate alternative green roof designs. Use of this logical and explicit decision-analytic tool will provide structure to the overall green roof evaluation process and will help installation managers to make informed and appropriate roof choices for a specific building.

**6 Environmental Monitoring using a Probabilistic-Possibilistic Approach** M. Ragheb, Univ of Illinois at Urbana-Champaign, Dept of Nuclear, Plasma and Radiological Engineering. An approach for Environmental Monitoring through a coupled Probabilistic-Possibilistic formulation is presented. Probability density functions based on sensors data, measurements and observations are coupled to membership functions, based on numerical or statistical modeling and defining the desired operational levels of the associated variables in the form of an information granule:  $g = (X)$  is  $(Y)$ , where  $X$  is a measurable or observable quantity or random variable subject to the laws of probability theory, and  $Y$  is a linguistic variable, semantic or agreed-upon meaning; quantifiable by simulation, modeling or expert opinion and subject to the laws of possibility theory. Examples of  $g$  can be the propositions:  $g_1 = (\text{radionuclide concentration})$  is (excessive),  $g_2 = (\text{radiation level})$  is (tolerable). Use of the Algebra of Logic allows a description of the overall environmental or ecological system operation and the connections among its subsystems in the form of an Operational Tree as the complement of a Fault Tree. In the definition of  $g$ ,  $X$  is represented by a probability density function (pdf)  $p_X(u)$ , and  $Y$  is a fuzzy variable represented by a membership function  $\mu_Y(u)$ . The Performance Level,  $PL$ , or the degree of truth of the proposition  $g$  can be estimated as the mathematical expectation of the observable  $X$ , as a value over the unit interval  $[0,1]$ . For continuous and discrete functions it can be estimated as:  $PL = \int \mu_Y(u) p_X(u) du$ ,  $PL = \sum \mu_Y(u) p_{Xi}(u)$ . Estimation of the performance levels of the



subsystems as well as the overall system allows for objective monitoring and assessment of its status. Evolution of effective monitoring would include learning from incidents, evaluation and correction of process safety, and the development of methodologies for anticipating and predicting accidents from the observation of their precursors and thus avoiding their occurrence rather than reactively responding to them.

#### 7 Relative Risks of Radionuclide Releases: Nuclear vs. Coal-Fired Power Plants

**S. Zemba**, Cambridge Environmental Inc. Comparisons of the environmental impacts of power generation alternatives are multi-dimensional, and can involve difficult comparisons of very different types of effects (e.g., climate change from due to emissions from fossil-fuel facilities, potential large-scale radionuclide releases from nuclear power plants, and ecological effects of hydro-electric projects). However, even “apples to apples” comparisons are sometimes ignored because some impacts are not widely recognized. Coal-fired power plants, responsible for a large share of worldwide electricity generation, emit radionuclides to the atmosphere in stack emissions, but do not garner the considerable attention focused on accidental releases of radioactivity from nuclear power plants. Coal contains trace levels of various radionuclides; when coal is combusted in power generation facilities, it creates a source of radionuclide emissions to the atmosphere which emissions then deposit and accumulate in soil. To compare, the effects of these emissions with the effects of more widely recognized atmospheric releases of radionuclides, our presentation will build on a detailed risk assessment of soil contamination found in the aftermath of the 1986 Chernobyl accident, using the “no action” exposure estimates based on Cs<sup>137</sup> uptake into agricultural products. Comparative estimates of radioactivity releases, combined with consideration of land use patterns, food production data, and other information will then be used to extrapolate the Chernobyl study to make order-of-magnitude estimates of human health risks associated with the recent accident at the Fukushima nuclear power plant. We will then compare these risks with cumulative risk estimates constructed for long-term emissions from a hypothetical conventional coal-fired power plant, based on available emissions information from the literature and the application of multi-pathway risk assessment methods, including air dispersion and deposition modeling, soil mixing and accumulation, and uptake into agricultural products from soil.

#### 8 We're Still Cleaning Up After the Manhattan Project: Lessons Learned Some Seventy Years Later

**K.G. Keil**, S.M. Bousquet, US Army Corps of Engineers, Buffalo District. The Formerly Utilized Sites Remedial Action Program (FUSRAP) has its origins in the Manhattan Project, when, in 1942, the US Army Corps of Engineers began development of the first atomic bomb. At that time, the Federal Government contracted with private industries, mostly in the northeastern US, to perform the uranium extraction, refining, and milling necessary to make an atomic weapon. It was not until several years later, after World War II ended, that these functions were transitioned to newly-created national laboratories. In the meantime, in the haste and secrecy of the war effort, the radioactive by-products of this weapons production began to leave its footprint on the industrial sites and the surrounding environment. Today, operating under FUSRAP and the CERCLA principles, the US Army Corps of Engineers is responsible for addressing residual contamination at 22 of these sites, 11 of which reside within the Buffalo District's boundaries. Using case studies and examples from these sites, this presentation will explore the time, effort, and cost incurred while characterizing and communicating environmental impacts and human health risks from the residual radiation to the surrounding public and stakeholders. In addition, the time, effort, cost, and risk reduction involved in remediating these sites to currently acceptable standards will be explored. Questions addressed will include: For those sites which have undergone public health assessments, dose reconstructions, or cancer surveys, do these other evaluation tools corroborate or contradict the CERCLA risk assessments used to make remedial action decisions conducted for the site, or risk perception of the site? What are the costs and benefits to human health and the environment in characterizing and remediating each of these sites? How are environmental impacts and benefits weighed vs. human health issues in the cleanups? What are the relative risks across the sites, and how does that impact priority of cleanup among the sites? What are the real vs. perceived risk drivers at the site, and how does risk perception affect the cleanup? What is reasonable future land use, and how does that impact cleanup decisions? Who (or what) benefits most from these cleanups?

#### 9 A SETAC Pellston Workshop on the Risks from Pesticides to Pollinators: State of the Science

**T. Steeger**, USEPA, Environmental Fate and Effects Division; F. Streissl, European Food Safety Authority (EFSA), PRAPeR Dept; A. Alix, Ministry of Agriculture, DGAL – SPRSP – SDQPV – BRMMI; T. Moriarty, USEPA; D.L. Fischer, Bayer CropScience, Global Development North America, Dept of Ecotoxicology; C. Lee-Steere, Sole Trader; R. Fischer, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL); M. Miles, Dow Agrosciences, Field Effects and Exposure; R. Johnson, Univ of Nebraska – Lincoln, Dept of Entomology; M. Fry, American Bird Conservancy; C. Brittain, Leuphana Univ of Lüneburg, Institute of Ecology and Environmental Chemistry. In recent years, a number of countries have reported declines in native and managed pollinators. Modern crop management practices, which often include the use of plant protection products or pesticides, have been identified as a potential factor responsible for these declines. To ensure the safety of pesticides regulatory authorities have adopted a number of measures, including requiring an assessment of the potential impact of these products on a broad range of taxa in the agricultural environment. Included in these assessments are the potential impacts of pesticides to terrestrial arthropods such as pollinating insects and honey bees (*Apis mellifera*) in particular. Consistent with the objectives of the Pellston workshop, participants considered risk assessment strategies for supporting a regulatory decision-making and risk management process for assessing the risks of pesticides to pollinators. The risk assessment process is typically tiered, iterative, and consists of three phases: problem formulation, analysis (exposure assessment and effects assessment) and risk characterization. This presentation will provide a broad overview of the risk assessment process employed by regulatory authorities globally in support of their decision making.

#### 10 Exposure Characterization – Report of the Exposure Workgroup from the SETAC Pellston Workshop on Assessing the Risk of Pesticides to Pollinators

**J.D. Wisk**, BASF Corporation, Ecotoxicology; J. Pistorius, Julius Kuhn-Institut, Institut für Pflanzenschutz in Ackerbau und Grünland; M. Beevers, California Agricultural Research, Inc.; R. Bireley, California Dept of Pesticide Regulation; Z. Browning, Browning's Honey Company, Inc.; M. Chauzat, French Agency for Food, Environmental and Occupational Health Safety; A. Nikolakis, Bayer CropScience; J. Overmyer, Syngenta Crop Protection, LLC, Ecological Sciences, Americas; R. Rose, United States Dept of Agriculture, Animal and Plant Health Inspection Service; R. Sebastien, Health Canada Pest Management Regulatory Agency; B.E. Vaissiere, French National Institute for Agricultural Research; M. Vaughan, Xerxes Society for Invertebrate Conservation. In the Workshop exposure workgroup, participants reviewed the various ways that honey bees (*Apis mellifera*) and non-*Apis* bees can be exposed to both non-systemic and systemic pesticides. Techniques that are employed to measure pesticide residues in matrices relevant for assessing bee exposure were summarized, and higher-tier semi-field and field study designs for refining bee exposure assessments were discussed. For tier 1 contact exposure estimates to foliar-applied products, published insect residue data can be used to develop an exposure nomogram where exposure estimates can be calculated based on application rates. For predicting oral exposure for sprayed products, participants proposed forming an industry coalition to compile pollen and nectar residue data from both published and proprietary studies to develop a nomogram that can be used to predict concentrations in pollen and nectar based on field application rates. Recognizing the limited field pollen and nectar residue data available for systemic compounds applied as seed treatment coating or soil applications to develop exposure models, participants recommended the proposal by the ICPBR for a default value of 1 mg/kg in pollen and nectar, as a Tier 1 point estimate of exposure for a screening-level assessment. Semi-field or tunnel tests using bee-attractive crops like Phacelia, oilseed rape, mustard or buckwheat are recommended to refine the oral exposure assessments for honey bee colonies to both systemic and non-systemic products sprayed on foliage. Semi-field studies are also recommended for assessing exposure to systemic pesticides used as seed dressings or applied as soil treatments. However, the actual crop being assessed should be used, as there may be different rates of uptake, distribution and metabolism of a compound in different plant species. Semi-field and field exposure studies can also be conducted using solitary nesting non-*Apis* bees such as blue orchard bees (*Osmia lignaria*) or alfalfa leafcutter bees (*Megachile rotundata*). Studies conducted with *Megachile* can also be used to evaluate potential dermal and/or oral exposure via contaminated nesting material. For assessing exposure to sprayed pesticides or systemic pesticides used as a



seed treatment, or applied as a soil treatment or trunk injection, field studies can also be conducted with the above non-*Apis* species to evaluate worst-case exposure because of the limited foraging range of these species.

### 11 Laboratory Assessment of Pesticide Hazards to Honey Bees and

**Non-*Apis* Bees** J. Pflugfelder, Agroscope Liebefeld-Posieux ALP; J. Frazier, Penn State Univ; P. Aupinel, INRA, Unite experimentale d'entomologie; P. Bachman, Monsanto Company; A. Decourtye, INRA, Lab Neurobiologie; C. Scott-Dupree, Univ of Guelph, School of Environmental Sciences; A. Dinter, Dupont de Nemours Deutschland; J. Ellis, Univ of Florida, Entomology; V. Grim, Hemholtz Centre for Environmental Research, Ecological Modeling; Z. Haung, Michigan State Univ, Entomology; R. C F Nocelli, CCA-UFSCar, Ciencias Biologicas; H. Thompson, Central Science Lab; B. Warren-Hicks, Consultant. For the risk assessment of pesticides to honeybees the laboratory assessment of hazards is the first step in a tiered testing approach. Tier 1 testing is conducted under controlled conditions with defined criteria on groups and individual bees and larvae to yield statistically valid determinations of the intrinsic activity of active ingredients. These tests produce an LD50 for acute contact and topical exposure, NOEC for chronic toxicity to adults and to bee larvae as the basis for a differentiated risk assessment if the product is a foliar spray or a systemic pesticide. Extrapolating these effects observed on individual bees to effects on the whole colony remains a major challenge. We suggest harmonizing the test procedures that are well developed, along with acknowledging an urgent need for the adaptation of new methods and provide perspectives on methods requiring further development and research. Extending similar tests to non-*Apis* bees requires further protocol development and standardization, along with agreements on which species offer the most advantages for laboratory, semi-field, and field testing. Sub-lethal impacts of pesticides on adults and larvae of both *Apis* and non-*Apis* bees are being documented in the scientific literature, but we still do not know to which extent *Apis mellifera* can serve as a surrogate. This needs to be defined and the development of a tiered test system for non *Apis* species requires significant effort and is a high research priority.

### 12 A SETAC Pellston Workshop on the Risks from Pesticides to Pollinators: Report of the Risk Assessment Breakout Group

A. Alix, Ministry of Agriculture, DGAI – SPRSPP – SDQPV – BRMMI. The intent of the global SETAC Pellston workshop was to bring together the best available science regarding exposure and effects assessment methodologies for honey bees (*Apis mellifera*) and non-*Apis* bees and to provide specific recommendations for determining the potential risk to insect pollinators from pesticide products in a cost effective way. The workshop intended to characterize exposure routes and produce related tiered standardized test method(s) to assess exposure from systemic and non-systemic pesticide products to bee species. Hazard endpoint(s), applicable to risk assessment for the honey bee, and tiered standardized test method(s) to consistently identify and measure hazard endpoint(s) were defined. A risk assessment process that can serve at both a screening-level and as a basis for more refined assessments where needed was proposed. The fourth objective of the workshop was to evaluate information on risk of pesticides to non-*Apis* bees. The resulting risk assessment process is predicated on first defining protection goals, and then identifying the appropriate assessment endpoints, i.e., explicit expressions of the actual environmental value that is to be protected, which can be used toward evaluating those goals. In developing this risk assessment process, breakout group members relied heavily on recommendations provided by other workshop breakout groups on exposure assessment and effects (hazard and field) assessment. The ratio of exposure to effects remains the basis of the risk assessment process defined in this section and is consistent with the regulatory processes reflected at the Pellston workshop. The risk assessment process then progresses towards higher tiers, which aim at evaluating the potential impact in the field, under more realistic exposure conditions. The highest tier approaches, i.e., full field studies, are intended to reproduce expected conditions of exposure for bees relative to how products will ultimately be used. These studies will be used to characterize the potential level of risk and identify the choice of risk management measures, if needed.

### 13 Measuring Pollinator Exposure to Imidacloprid from Hardwood Trees Treated to Control the Asian Longhorned Beetle

J. Pettis, USDA/ARS Bee Research Lab ; J.D. Johnson, Univ of Maryland, Dept of Toxicology; P. Lewis, USDA,APHIS,PPQ; R. Rose, USDA,APHIS,PPQ,EDP. Animal and Plant Health Inspection Service (APHIS) has undertaken an eradication program to remove and prevent the further spread of

Anoplophora Glabripennis, the Asian longhorned beetle (ALB) in New York City. Several hardwood species can serve as host for ALB and imidacloprid (IMI) is used to treat uninfested trees in ALB infested areas in part to save the trees and prevent expansion of beetle range. In Staten Island, trunk injections of imidacloprid, a neonicotinoid, were used because the water table is high and water contamination by the soluble pesticide could be significant, whereas in the drier soils in the boroughs of Queens and Brooklyn soil injections of the twenty+ susceptible species of trees were more appropriate. We chose red maple, *Acer rubrum*, as our target tree as pollinators use the nectar and pollen of this tree during the early spring and it is a dominant species in this area. Imidacloprid and six of its metabolites, were monitored in red maple flowers, pollen collected by honey bee hives placed in the immediate areas, and red maple leaves over a 6-year treatment protocol to assess the environmental presence of the pesticide as a potential threat to foraging honey bees, a non-target species. Results show that soil injected trees had higher amounts of IMI in flowers than trunk injected trees. IMI partitioned mostly to the leaves as expected since this systemic pesticide moves through the xylem (Sur and Stork, 2003) but flowers had detectable amounts suggesting that phloem transport also takes place but at a slower rate. Male flowers had higher amounts than female flowers. Pollen loads collected by honey bees placed at the test sites had detectable levels of imidacloprid. Repercussions on honey bee health will be discussed.

### 14 Examples of Higher-tier Studies – Semifield Studies

A. Schur, Eurofins-GAB GmbH, Ecotoxicology, Eurofins Agrosience Services; I. Tornier, Eurofins Agrosience Services. The existing European guideline OEPP/EPPO No. 170 (4) for honey bee (*Apis mellifera* L.) testing of plant protection products are used as basis for tests under laboratory, field and semi-field conditions. In the presentation methods and results of tests under semi-field conditions will be summarized and discussed. The OEPP/EPPO No. 170 (4) guideline was mainly developed in the northern part of Europe. In the past years, higher tier tests were more adapted to the needs of testing pesticides in the southern European zone and in different kinds of crops. However, within such tests different parameters, such as bee mortality, flight intensity in the crop, behaviour of the bees and the conditions of the colonies and brood development are assessed before as well as after the application of a plant protection product. Within the presentation different kinds of methods according to the guideline will be presented which are used in different kinds of crops, e.g., *Phacelia tanacetifolia*, winter oil-seed rape, citrus, melon, peach as well as in the northern, central and southern part of Europe. In addition, results will be shown generated within the studies for the water-treated or untreated control, the toxic reference treatment (dimethoate) in comparison to different kinds of test compounds. The results of the mortality, flight intensity and bee colony assessments shows, that there is a wide range in the results assessed in different studies, but also within the replicates of the same treatment group of one study. This causes difficulties in the evaluation of results and in the risk assessment of a compound. Beside standard tests including the above mentioned parameters, studies are often combined with the assessment of residues in e.g., nectar and pollen, detailed assessments on behaviour and sub-lethal effects, detailed assessments on brood development and the comparison of different treatment scenarios (e.g., pre-flowering application, evening application compared).

### 15 Large-scale Field Study to Assess Side-effects of the Insecticide Spirotetramat to Honey Bee Colonies Under a Realistic Field Scenario in a Melon Crop

T. Stadler, Laboratory of Environmental Toxicology-IMBECU-CONICET, Institute of Medicine and Experimental Biology; H.J. Schnorbach, Bayer CropScience BCS AG-D-AD; C. Maus, Bayer CropScience BCS AG-ACO-M-PS. Spirotetramat (Movento®) is an insecticide which acts as a lipid biosynthesis inhibitor that is being used on a great variety of crops to control a broad spectrum of sucking pests. An intrinsic larvicidal potential of the compound was shown in lower tier tests with artificial exposure conditions (confinement or feeding of very high concentrations). In order to achieve higher-tier data to conduct a scientifically sound risk assessment of actual field use, a replicated large-scale field trial design was developed to test for potential side effects under realistic use conditions of the product. The study was conducted in western Argentina. Two application scenarios were tested. In particular brood development was evaluated, but other endpoints such as mortality, foraging activity and hive weight development were also assessed. Nine field plots at least 3 km apart were randomly assigned to receive either treatment scenario 1 (4 x 75 g a.s./

ha), treatment scenario 2 (2 x 88 g a.s./ha) or control (water) applications (i.e., 3 replicates per treatment group). Two bee hives were set up per plot. Each hive contained a queen-right colony of ca. 18,000 bees. The hives were set up on the plots 2.5 weeks before the first brood assessment. Four further weekly in-field brood assessments were conducted, starting 5 days after the first application. Study duration was nine weeks. No adverse effects were recorded during the study. The comb area containing brood of all stages fluctuated in the control as well as in treatment groups on the different assessment days, indicating the typical natural variability of this endpoint. No treatment-related effects on brood and colony strength were found, nor were there any treatment-related differences in mortality. Likewise, foraging activity, nectar and pollen storage and hive weight development were unaffected in both treatment groups. It is therefore concluded that the tested uses of spirotetramat on melons are reasonably safe to honey bees. The results of the study reveal that replicated honey bee field studies conducted under practical agricultural conditions provide an excellent tool to reliably assess the risk of plant protection products to bees, as they provide maximum realistic insights in a holistic approach that monitors the exposed bee colonies along with all relevant environmental factors influencing its health, and are at the same time capable to overcome the natural variability of the testing system.

**16 Do Reported Levels of Pesticides in Hive-stored Pollen Pose a Serious Risk to Honey Bees?** D.L. Fischer, Bayer CropScience, Global Development North America, Dept of Ecotoxicology; C. Henderson, Univ of Montana, Bee Alert Technologies, Inc. Several recently published studies have used multi-residue analytical methods to screen samples of pollen stored in bee hives for presence of pesticides. These papers have sometimes suggested the levels and frequency of pesticide detections are alarming and emphasized that because residues of multiple pesticide active ingredients commonly co-occur in samples, synergistic toxic effects may be occurring. The occurrence of systemic pesticides in hive samples is sometimes emphasized as reason for additional concern. We applied risk analysis methods for honey bees and pesticides recently developed by the ICPBR Bee Protection Group to evaluate whether recently published measurements of pesticide levels in hive-stored pollen pose a serious risk to honey bees. Our analysis indicates the reported chemical exposures likely pose minimal risks to honey bees. This was true for the individual chemicals assessed one at a time as well as for a worst-case theoretical mixture containing a 95<sup>th</sup> percentile concentration for every chemical in the respective data sets. Contrary to what has often been assumed, our analysis revealed that systemic pesticides are found less frequently and at lower levels in hive-stored pollen than non-systemic pesticides, and risk estimates for systemic compounds were if anything lower than for non-systemic pesticides. Our analysis indicates that one should not assume a serious risk exists based on the mere presence of residues in bee hive samples. A risk analysis, such as that presented here, needs to be undertaken before conclusions about risk can be drawn.

**17 In Vivo and In Vitro Estrogenicity and GC/MS/MS and LC/MS/MS Quantification of Estrogens in Aqueous Mixtures of Raw and Pelletized Poultry Litter** L.T. Yonkos, Univ of Maryland, Wye Research and Education Center; D.J. Fisher, Univ of Maryland, Wye Research and Education Center; E.A. Friedel, Univ of Maryland, Wye Research and Education Center; V.S. Wilson, USEPA, ORD, NHEERL, TAD, Reproductive Toxicology Branch, USEPA, Office of Research and Development; S.R. Hutchins, USEPA, ORD, GWERD; J.M. Lazorchak, USEPA, Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; T.V. Reddy, USEPA, NERL; N.W. Shappell, USDA-ARS-RRVARC, Biosciences Research Lab, USDA-ARS; P.A. Van Veld, Virginia Institute of Marine Science; R.C. Hale, Virginia Institute of Marine Science, Environmental and Aquatic Animal Health. Abundance, degradation, and bio-activity of estrogens were examined in aqueous solutions of poultry litter from three Delmarva broiler integrators, a pelletized litter sample, a biosolids sample from a regional WWTP, and an estrone (E1) positive control allowed to stand static for 28 days. Litter and biosolids samples were generated to be analogous to organically fertilized agricultural runoff. Estrogens, 17 $\beta$ -estradiol (E2) and E1, were quantified in aqueous samples via GC/MS/MS and LC/MS/MS at day 0, 9, and 28. Likewise, estrogenicity was investigated using a pair of in vitro assays (E-screen and K<sub>bluc</sub>) on days 0, 9, and 28 and in vivo by exposing mature male fathead minnows (*Pimephales promelas*) continuously (day 0 – 9) to investigate vitellogenin (Vtg) induction (plasma protein and liver Vtg mRNA). All three raw litter samples showed a significant increase in E1 and E2 between day

0 and day 9 before decreasing by day 28. In vitro estrogenicity showed a corresponding increase to day 9 with EEQ (E2 equivalent) levels paralleling E2 concentrations measured via GC/MS/MS. All litter samples (raw and pelletized) induced Vtg with plasma protein and mRNA concentrations correlating strongly with peak (day 9) E2 concentrations. Increases in estrogens between day 0 and day 9 are likely explained by microbial deconjugation of glucuronide and sulfate groups to produce native/bioactive E1 and E2. Results indicate that fecal estrogens can persist and estrogenicity actually increase in aqueous solutions days to weeks after introduction. Results and implications to natural waters will be discussed.

**18 Sex Differentiation as a Target of Endocrine Disrupting Compounds in Early Life Stage Fathead Minnows (*Pimephales promelas*)** J.K. Leet, Purdue Univ, Forestry and Natural Resources; J.J. Amberg, United States Geological Sciences, Upper Midwest Environmental Sciences Center; A. Olmstead, US Environmental Protection Agency, Mid-Continent Ecology Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; L.S. Lee, Purdue Univ, Dept of Agronomy Crop, Soil & Environmental Sciences; M.S. Sepulveda, Purdue Univ, Forestry & Natural Resources and School of Civil Engineering, Purdue Univ, Forestry & Natural Resources. The occurrence of endocrine disrupting chemicals (EDCs) in concentrated animal feed operation (CAFO) waste, and the potential effects of these chemicals on aquatic ecosystems have been of recent concern. There is evidence that exposure to EDCs during enhanced windows of sensitivity can lead to alterations in fish sex differentiation. Fathead minnows (*Pimephales promelas*) are commonly used as a model fish species in endocrine disruption studies, however limited knowledge exists on molecular pathways associated with sex differentiation in early life stages of this species. A method has been developed to determine genetic sex in early life stage fathead minnows by use of a sex-linked DNA marker. Changes in the expression of genes important in sex differentiation (cyp19a, sox9, dmrt1) could be used as a way to evaluate effects of EDCs on gonadal development and sex differentiation. For example, cyp19a expression doubles in females from 10 to 15 days post hatch (dph) and is 6 fold higher by 20 dph, but does not significantly change in males during this time. This period in development has also been found to be a sensitive window for fathead minnows exposed to EDCs. We hypothesize that the expression of cyp19a and other genes involved in sex differentiation and gonad development will be altered in a sex specific manner when fathead minnows are exposed to androgens, estrogens, or mixtures of EDCs during sex differentiation. Preliminary data from our lab show that fathead minnow embryos exposed to trenbolone (a potent synthetic androgen) respond with significant alterations in expression of such genes. Evaluation of these molecular markers in combination with this new method of sex identification is being used for developing efficient tools to evaluate sex specific responses of EDCs on early life stage fish. These tools will help us gain knowledge of the molecular pathways controlling sex differentiation in fathead minnows and how EDCs may alter these processes.

**19 Endocrine-disrupting Potency of By-products in Flame Retardants** P. Leonards, Institute for Environmental Studies, VU Univ, Chemistry & Biology, VU Univ, Institute for Environmental Studies; S. Brandsma, VU Univ, Institute for Environmental Studies. Some brominated flame retardants (BFRs) have unintended negative effects on the environment and human health (e.g., endocrine effects). Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, some are very persistent, and some show serious toxicological effects such as endocrine disruption. During the last decade an increasing number of reports have presented evidence of these negative effects caused by specific BFRs. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects and exposure are lacking. The European Commission-funded project ENFIRO investigates halogen-free substitution options for specific BFRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and a life cycle assessment. In total 15 halogen-free flame retardants (HFFRs), consisting of metal-, organic-, and nano-based FRs, as substitution options for decaBDE, TBBP-A, and brominated polystyrenes were selected. These HFFRs were assessed in a toxicity screening to provide information on the hazard characterization. Toxicity profiling of the 15 HFFRs and 3 BFRs was carried out with a battery of in vitro assays (e.g., cytotoxicity, thyroid hormone disruption, estrogenicity, androgenicity, mutagenicity) and showed that

three organic HFRs had estrogenic potency. These HFRs were chemically fractionated on polarity and the fractions re-tested in the estrogenic in vitro assay. Compounds present in the active fractions were chemically identified with LC-MS, LC-ToFMS and GC-ToFMS, and showed that not only the flame retardants but also by-products in the flame retardant product were responsible for the estrogenic potency. In conclusion, the combination of mode of action screening and chemical identification is a powerful tool to study and identify potential endocrine disrupting chemicals.

## 20 Regulation of the Transcriptional Control of the Largemouth Bass Vitellogenin Receptor by Androgens and Waste Water Effluent Mixtures

**G.A. Dominguez**, Univ of Florida, Center for Environmental and Human Toxicology; **K.J. Kroll**, N. Denslow, Univ of Florida, Physiological Sciences; **T. Sabo-Attwood**, Univ of Florida, Dept of Environmental and Global Health. There is increasing concern surrounding the presence of androgenic compounds in environmental settings such as effluents of industry and waste water treatment (WWT) facilities. Although numerous investigations have revealed the physiological effects on female fish, such as decreased fecundity, reduced egg size and spawning events, exposed to androgen-related and – suspected compounds minimal studies have explored specific mechanisms of action. While major efforts have focused on estrogen disruption in teleosts through estrogen-driven mechanisms of vitellogenin (VTG) production in the liver, fewer studies have focused on pathways that control yolk accumulation into growing oocytes, a process essential for successful reproduction. One gene important to proper egg development is the vitellogenin receptor (VTGR) as it sequesters circulating vitellogenin (VTG) into developing oocytes. To begin to address the molecular mechanisms that control the transcription of this gene, we cloned the full length cDNA and partial promoter for the VTGR of largemouth bass. Using quantitative real-time PCR we show maximal expression of the VTGR in gonadal tissues during primary oocyte stages of growth whereas the levels decrease during VTG stages of oocyte development when maximal VTG synthesis is observed. Further analysis using reporter gene assays driven by a partial VTGR promoter reveal activation by androgens including 11-ketotestosterone (11-KT, 100 nM) and trembolone (100 nM) whereas estrogens failed to produce a response. Furthermore, extracts collected from a waste water effluent holding pond also showed similar activation by comparison to 11-KT and trembolone. Inhibition of these androgenic responses was diminished with co-exposure to the androgen receptor antagonist flutamide. Outcomes of these studies reveal, for the first time, that androgens, not estrogens, control the expression of VTGR in female fish which is mediated through androgen receptors and may be a target of complex environmental mixtures. These mechanisms may also explain endpoint effects observed in female fish exposed to androgens and androgen-containing mixtures and highlight the importance of elucidating the role of androgens in females and endocrine disrupting capacity of proper egg development.

## 21 Intersex Fish Influenced by Factors Other than Municipal Wastewater Effluent

**K. Kellock**, Univ of Georgia, Interdisciplinary Toxicology Program; **C. Jennings**, P. Ely, USGS Georgia Cooperative Fish and Wildlife Research Unit; **B. Trushel**, USGA Georgia Cooperative Fish and Wildlife Research Unit; **R. Bringolf**, Univ of Georgia, Interdisciplinary Toxicology. Recent reports of intersex fish (males with oocytes in their testicular tissue) in water bodies around the world have stimulated widespread concern about the effects that chemicals are having in the environment. Estrogens and estrogen mimics in the aquatic environment are known to induce intersex and other forms of endocrine disruption in fish. Intersex fish can have decreased sperm production, decreased sperm motility and decreased fertilization success compared to histologically 'normal' male fish. Recently, a high incidence of intersex fish were reported in some rivers in the southeastern US but to date, a systematic evaluation of the extent, severity, and causes of intersex fish has not been completed in Georgia. Therefore, our objectives were to (1) assess intersex condition in black bass collected from rivers and impoundments across Georgia, and (2) determine estrogenic potency (a measure of the estrogens and estrogen-like substances) of surface waters where fish were collected. Fish (N>15) and water samples were collected near municipal wastewater effluent discharges in each of four rivers and from a river that receives no major wastewater effluent discharges. Fish were also sampled from 11 impoundments with no major wastewater inputs to determine if a natural 'background' rate of intersex could be established for fish from relatively unpolluted water bodies. Gonads from all fish were examined and the incidence and severity of the intersex condition were compared among sites.

The overall percentage of intersex for male bass collected in rivers was 11% while 52% of male fish collected from impoundments were intersex. Incidence of intersex varied substantially (0-100%) among impoundments and surface area of the impoundment was a strong predictor of incidence. Severity of intersex also varied among impoundments but was not predicted by surface area. Potency of estrogens in surface waters was determined by use of an in vitro yeast-based reporter gene (YES) assay. The specific mechanism(s) causing intersex remain unknown, but the high incidence of intersex males in some impoundments suggests that factors other than municipal wastewater are involved.

## 22 Mode of Action-based Screening of Endocrine Disrupting Chemicals in Wastewater Effluents

**R. Marfil-Vega**, USEPA, ORISE Research Participant to the National Risk Management, Univ of Cincinnati; **M.A. Mills**, USEPA, National Risk Management Research Laboratory; **S.F. Nakayama**, National Institute for Environmental Studies, Center for Environmental Health Sciences; **M.W. Hornung**, **M. Gilbert**, **K.M. Crofton**, USEPA, National Health and Environmental Effects Research Laboratory; **K.B. Paul**, The Hamner Institutes for Health Sciences, Institute for Chemical Safety Sciences, Integrated Systems Toxicology Division, NHEERL, ORD, USEPA; **K. Tadele**, USEPA, Student Services Contract at National Risk Management Research Laboratory. Endocrine disruption encompasses the disturbance of different metabolic pathways, comprising the nuclear receptors for steroid and thyroid hormones, among many others. Emerging contaminants both from natural and industrial origin, such as the steroid and thyroid hormones themselves, commercial and industrial chemicals, and pharmaceutical and personal care products (PPCPs) that are known to be endocrine disruptors are often detected in environmental samples. An ongoing multi-laboratory and multi-disciplinary effort to monitor and characterize emerging contaminants in wastewater treatment plant effluents is aimed at determining the endocrine disrupting activity of these effluents, and the specific chemicals that may underlie these effects. Synthetic progestagens, which are agonist of the progesterone receptor, are used for oral contraception, alone or in combination with synthetic estrogens. Combined formulations present a higher amount of progestagens than estrogens; therefore, there is an elevated potential risk for progestagens to reach the environment from wastewater treatment plants discharges. In regard to the thyroid hormone, the complexity of its homeostasis allows for structurally diverse chemicals that may disrupt its different biological endpoints. Known thyroid disruptors, such as perchlorate, thiouraciles, thiocyanates, PCBs, dioxins, furans, and pesticides, are commonly found in the environment. But other chemicals that may be present in wastewater effluents (thyroid hormones, benzothiazoles and some PPCPs) need to be considered to account for the total thyroid disruption activity of these samples. In the current work, we will be presenting the initial results from monitoring progestagens and selected thyroid disruptors in wastewater effluents, in combination with the chemical quantification of the disruption potency of the effluents against a battery of in vivo and in vitro bioassays (i.e., thyroid gland explant culture assay, thyroid peroxidase inhibition assay, and human pregnane-X and progesterone cell-based nuclear receptor reporter assays).

## 23 Application of the Transthyretin Binding Assay in Effect-Directed Analysis of Sediments: Identification of Thyroid Hormone Disrupting Compounds

**M. Lamoree**, Institute for Environmental Studies, VU Univ, Chemistry & Biology, Institute for Environmental Studies, Chemistry & Biology; **T. Hamers**, Institute for Environmental Studies, VU Univ; **E. Simon**, BioDetection Systems; **P. Leonards**, Institute for Environmental Studies, VU Univ; **J. Weiss**, Joint Research Centre – European Commission. In Effect-Directed Analysis (EDA), (in vitro) bioassays, sample fractionation techniques and chemical analysis are used in an iterative manner in order to identify the compounds that are responsible for an observed biological/toxicological effect. In EDA studies, various bioassays have been implemented that have an endpoint related to endocrine disruption, with the emphasis on estrogenic and androgenic effects. To extend the scope of EDA, we have used the radioligand T4\*-TTR binding assay (shortly, TTR assay) to determine the thyroid hormone like (TH-like) activity to direct our fractionation for the identification of unknown thyroid hormone disrupting environmental contaminants. From a human and animal health perspective, the thyroid function is vital for the normal development of the central nervous system of the fetus. To assess what classes of environmental toxicants are capable of interfering with the thyroid hormone system, an inventory of the different classes of compounds with reported TH-like activities was



made. Compounds that are known to influence the thyroid system are, among others, hydroxylated polybrominated diphenyl ethers, hydroxylated polychlorinated biphenyls and triclosan. More recently, also polyfluorinated compounds were shown to have moderate TH-like activities. In the framework of the EU funded Modelkey project we have carried out EDA studies at several locations connected with European river systems using a variety of bioassays. Sediment extracts were fractionated using sequential reversed and normal phase liquid chromatography. In the course of this project, we identified several different classes of environmental toxicants in fractions of the responsive sediment extracts, by conventional gas chromatography coupled with mass spectrometric detection as well as liquid chromatography coupled with high resolution mass spectrometry. A selection of these compounds was tested in the TTR assay in order to assess whether they were responsible for the observed TH-like activity in the fractions of the sediment extracts.

**24 The Role of Sediment in the Bioavailability of Steroid Hormones 17 $\beta$ -Estradiol and Trenbolone** J. Sangster, Univ of Nebraska – Lincoln, Dept of Civil Engineering; S. Bartelt-Hunt, Univ of Nebraska, Civil Engineering; N. Conoan, Univ of Nebraska – Omaha, Dept of Biology; A.S. Kolok, Univ of Nebraska Medical Center, Environmental, Agricultural and Occupational Health. There is growing concern about the impact of steroidogenic compounds in aquatic systems. Steroids released into the environment have been shown to sorb to sediments in aquatic environments, however other studies have suggested that steroids can move through sediment beds, and that fish and other aquatic organisms can facilitate this mobility. In this study, we investigated the effect of sediment-associated 17B-estradiol and trenbolone on vitellogenin expression in fathead minnows, a ubiquitously distributed freshwater organism. Two natural sediments were selected to represent a diversity of soil properties, one predominantly clay and one predominately sand. Sediment contaminated with either trenbolone or 17B-estradiol was placed in a glass aquaria along with 20 sexually mature female (trenbolone) or male (17B-estradiol) fathead minnows. Both positive (aqueous steroid) and negative (lab water) control experiments were performed. Ten fish were randomly collected from each tank at 7 days with the remaining harvested at 14 days. Livers and gonads were collected from each individual and relative hepatic vitellogenin (Vtg) expression was quantified using ribosomal L8 as a normalization standard. After exposure to 17B-estradiol-contaminated sediments, male fathead minnows exhibited no inappropriate expression of Vtg relative to the negative control group regardless of the sediment type used. By comparison, female fathead minnows exposed to sediment-associated trenbolone did exhibit a reduction in Vtg expression relative to negative controls when the clay sediment was used. However, female fathead minnows exposed to trenbolone associated with sand exhibited no inappropriate expression of Vtg relative to the negative control. Taken together, we observed differential bioavailability for an estrogenic compound (17B-estradiol) and an androgenic compound (trenbolone) when associated with sediment. Additionally, differential bioavailability was observed for trenbolone depending on sediment type. Liquid chromatography tandem mass spectroscopy will be performed on sediment extracts and water samples obtained over the exposure period to evaluate steroid desorption and transformation over the exposure period.

**25 Adverse Outcome Pathways: Definition and Utilization** G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division. A challenge for both human health and ecological toxicologists is the transparent application of mechanistic (e.g., molecular, biochemical, histological) data to risk assessments. The adverse outcome pathway (AOP) is a conceptual framework designed to meet this need. Specifically, AOPs portray causal and predictive linkages between molecular-cellular disruption (initiation of a toxicity or disease pathway) and adverse outcomes of regulatory significance in individuals or populations. Collecting, analyzing and communicating toxicological data through the use of AOPs enhances aspects of risk assessments related to extrapolation of chemical effects across biological levels of organization, species and chemical structures. AOPs also provide a platform for assessing cumulative risk of chemical mixtures. This presentation will provide an overview of the AOP concept relative to other frameworks (e.g., mechanism/mode of action, toxicity pathways), review the outcomes of various meetings and workshops focused on the development and application of AOPs, and present an overview of utility of the concept in different risk assessment/regulatory scenarios. The contents of this abstract do not reflect USEPA policy.

**26 Establishing Adverse Outcome Pathways of Thyroid Hormone Disruption in an Amphibian Model** M.W. Hornung, USEPA, National Health and Environmental Effects Research Laboratory; S.J. Degitz, J.E. Tietge, US Environmental Protection Agency, Mid-Continent Ecology Division. The Adverse Outcome Pathway (AOP) provides a framework for understanding the relevance of toxicology data in ecotoxicological hazard assessments. The AOP concept can be applied to many toxicological pathways including thyroid hormone disruption. Thyroid hormones play a critical role in vertebrate morphological and neural development, and are important for maintenance of multiple physiological functions. Control of thyroid hormones is conserved across vertebrates via regulation by the hypothalamic-pituitary-thyroid axis (HPT). It has been well-established that disruption of the ability of the HPT axis to maintain normal levels of thyroid hormones can occur through exposure to various anthropogenic chemicals in the environment. Understanding the capacity of these chemicals to disrupt thyroid hormone levels and produce an adverse effect in the organism is critical for making sound risk assessment decisions. Furthermore, linking the initial molecular event in which a chemical interacts with the biological target to the processes leading to the adverse outcome, can provide a basis for developing predictive chemical structure activity relationships for thyroid hormone disruption. The molecular initiating events that can lead to decreased circulating thyroid hormone include inhibition of iodide uptake by the thyroid via the sodium-iodide symporter, inhibition of thyroid peroxidase which directly catalyzes the production of hormone, and induction of thyroid hormone metabolizing enzymes, including the thyroid hormone deiodinases. The amphibian provides an excellent model for developing AOPs that cover the various potential pathways to thyroid hormone disruption because amphibian metamorphosis is dependent upon thyroid hormone and is an easily quantified apical endpoint. The development and application of in vitro, ex vivo, and in vivo assays as they are applied to understanding disruption of thyroid hormone regulation in vertebrates will be presented. These assays include in vitro enzyme assays that measure the ability of a chemical to directly inhibit thyroid hormone synthesis, ex vivo assays that may be used to assess thyroid hormone release and metabolism, and final confirmation of an adverse effect with an in vivo metamorphosis assay. Developing a suite of AOPs for thyroid hormone disruption can provide an integrated set of mechanistic tools for identifying and verifying potentially active chemicals. This abstract does not necessarily reflect EPA policy.

**27 From Molecular Mechanism of Action to Ecological Risk Assessment: The Aryl Hydrocarbon Receptor and Avian Species Sensitivity to Dioxin-like Compounds** S.W. Kennedy, Univ of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre; R. Farmahin, Univ of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre; D. Crump, Environment Canada, National Wildlife Research Centre; G.E. Manning, Univ of Ottawa, Dept of Biology; S.P. Jones, Environment Canada, National Wildlife Research Centre; S.J. Bursian, Michigan State Univ, Dept of Animal Science; T.B. Fredricks, Monsanto Company; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; M.J. Zwiernik, Michigan State Univ, Dept of Animal Science; M.E. Hahn, Woods Hole Oceanographic Institution; S.I. Karchner, Woods Hole Oceanographic Institution, Biology. An important goal of environmental toxicology is the development of methods that allow prediction of the sensitivity of wild species to adverse effects of environmental contaminants. We are conducting studies to determine if it is possible to predict the sensitivity of any wild avian species to the embryotoxic effects of any 'dioxin-like' compound (DLC) based on a detailed molecular and biochemical understanding of how DLCs interact with the aryl hydrocarbon receptor 1 (AHR1). A series of laboratory studies were conducted using AHR1-dependent reporter gene expression assays, AHR1 binding assays, primary hepatocyte cultures and egg injection studies. The results of these studies, in combination with sequence information for the AHR1 ligand binding domain (LBD) of 75 bird species, were used to develop an approach for predicting the sensitivity of birds to polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs). The results indicate that the sensitivity of avian species to DLCs can be predicted from the AHR1 LBD genotype. This research represents an example of how the knowledge of the fundamental mechanisms of action of a class of environmental chemicals can be used to develop methods of use for environmental risk assessment.

**28 Building Chemical Categories for Ecotoxicological Endpoints** T. Schultz, Univ of Tennessee; R. Diderich, OECD. There is discontinuity between chemical and biological spaces so substances, which are "similar" in molecular structure, are often dissimilar in terms of their toxicity. This difference leads to difficulties in forming toxicologically meaningful groups, especially for chronic endpoints. Identification of a consistent pattern of toxic effects via a chemical category increases the confidence in the reliability of the results for all category members. However, this is predicated on a priori binning the chemical into the correct category. Thus, category formation is the key to category-based assessments. Most scientific and regulatory decisions are made using a weight of evidence approach using all available and relevant information. Using a weight of evidence approach requires the identification of information needs which are endpoint specific. The question becomes how does one identify a mechanistically plausible and transparent means of organizing information to characterize the weight of evidence and provide a basis for testable hypotheses for a chemical category. The Adverse Outcome Pathway (AOP) has been put forth as a way of building categories for complex endpoints by integrating the sciences that explain each important element of the complexity. This is more transparent and internally consistent to each endpoint as it can be the result of a causal chain of effects that are dependent on the chemical structure, the biological response processes for the species, sex and life stage, as well as the exposure scenario. AOP-based categories shift the emphasis away from statistical similarity to intrinsic chemical and biological activity. They aid in developing hypotheses which can be tested. They encourage expert discussion about chemical and toxicity mechanisms and modes of action. And, they enable all chemicals in the category to be assessed in a defensible manner when only a few members are tested. AOPs are useful in assessing ecological health where the focus is on the health of populations and not so much the individual, where protection of the population focuses on shorter-term survival and longer-term reproduction, and where protection of ecosystems are often based on surrogate species testing with the effects extrapolated to many taxa and species.

**29 Experimental Approaches to Systematic Discovery and Development Reproductive Adverse Outcome Pathways in Fish** D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; D. Bencic, USEPA, Office of Research and Development; T. Collette, USEPA, National Exposure Research Laboratory; S. Edwards, USEPA; D. Ekman, USEPA, National Exposure Research Laboratory; N. Garcia-Reyero, Jackson State Univ, Dept of Chemistry; J.M. Lazorchak, USEPA, Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; E.J. Perkins, US Army, Engineer Research and Development Center, Environmental Laboratory, Environmental Processes and Effect Division. Adverse outcome pathways (AOPs) are conceptual frameworks that portray causal and predictive linkages between key events at multiple scales of biological organization, providing connectivity between molecular initiating events at the cellular level (e.g., initiation of toxicity pathways) and adverse outcomes of regulatory significance (i.e., at the individual and/or population level). Proposals to make greater use of high throughput in vitro screening, quantitative structure-activity relationships, and other types of alternative data for screening, prioritization, and hazard assessment are dependent, in part, on the ability to identify major adverse outcome pathways, and develop associated assays and computational tools for predicting and ranking chemical hazards. This presentation will describe a program of research that was undertaken to systematically discover, characterize, and develop AOPs for reproductive toxicity in fish and other vertebrates. Specifically, it illustrates how hypothesis-based experimentation anchored to conceptual systems models can guide AOP development. It also examines ways in which toxicogenomic data (i.e., transcriptomics, metabolomics) can be used to accelerate discovery of novel AOPs and improve our understanding of AOPs in a dynamic systems context that considers feedback processes and intersections/interactions between multiple pathways. Although the examples will focus on AOPs for reproductive toxicity in fish, the concepts employed are broadly applicable to a wide range of other adverse outcomes and model systems. The contents of this abstract neither constitute nor reflect official USEPA policy.

**30 New Predictive Computer and In Vitro Based Tools in Ecological Risk Assessment for Faster and More Effective Prioritization of**

**Chemicals** T. Steeger, USEPA, Environmental Fate and Effects Division; M. Panger, USEPA, Environmental Fate and Effects Division. The US Environmental Protection Agency (USEPA) Office of Pesticide Programs (OPP) currently relies heavily on in vivo testing where data are largely based on measurement endpoints directly linked to reproduction, growth, and survival. OPP is committed toward increased use of in vitro methods and computer-based predictive models that allow more efficient use of resources and reduce the number of animals used in testing. The Adverse Outcome Pathway (AOP) concept spirited by the USEPA Office of Research and Development (ORD) Mid-Continent Ecology Division provides a framework for linking initial molecular or cellular effects measured by in vitro assays to an adverse outcome of relevance in risk assessment (i.e., effects seen in individuals or populations). This conceptual framework, once sufficiently developed, would better enable risk assessors to make use in vitro methods and information collected at multiple levels of biological information to estimate whole organism and population-level effects. Until libraries of data demonstrating linkages between molecular initiating events, key events and apical endpoints are developed, the utility of in vitro methods may be limited to identifying degradates of concern and possibly prioritizing the chemicals needing expedited reviews. The AOP concept though will lead to greater qualitative and eventually quantitative use of in vitro, high through-put (HTP) assays in risk assessment. This presentation will provide an overview of the current and future use of these tools in the ecological risk assessment of conventional pesticides.

**31 Impact of the Polychaete Worm *Nereis Diversicolor* on Mercury Speciation in Intertidal Mudflats** T. Sizmur, Univ of Reading, Soil Research Centre, Soil Science Dept; S. Edmonds, Acadia Univ, Biodiversity Research Institute; A. Godfrey, Acadia Univ; J. Canario, INRB IP/IPIMAR; A. Redden, M. Mallory, G. Gibson, N. O'Driscoll, Acadia Univ. Central Nova Scotia is a biological mercury (Hg) hotspot as evidenced by elevated Hg concentrations in organisms feeding at higher trophic levels. The Minas Basin in the Bay of Fundy, Nova Scotia is home to the highest tidal amplitudes in the world giving rise to extensive intertidal mudflats. Some areas of mudflats in the Minas Basin contain elevated concentrations of methyl mercury (MeHg), due to the availability of inorganic matter and to the reduction of Fe(III) and/or  $\text{SO}_4^{2-}$  during organic matter oxidation by sediment microorganisms. Recent observations indicate a change in the ecology of the mudflats leading to an increase in the abundance of polychaete worms such as *Nereis diversicolor*. *N. diversicolor* feed on organic detritus and microorganisms associated with surface sediments and live in permanent U- or Y-shaped burrows which they ventilate and oxygenate with fresh seawater, increasing the sediment-water interface by up to three times. Sediments were collected from four intertidal mudflats in the Minas Basin at Wolfville (MeHg: 569 pg g<sup>-1</sup>), Windsor (MeHg: 546 pg g<sup>-1</sup>), Hantsport (MeHg: 358 pg g<sup>-1</sup>) and Kingsport (MeHg: 448 pg g<sup>-1</sup>) and incubated 10 *Nereis diversicolor* (collected from the Wolfville site) in microcosms containing 300 g of sediments alongside worm-free control microcosms for 14 days. Hg(II) and MeHg concentrations were determined in samples taken from surface and bulk sediments of all treatments and sediments from the oxygenated burrow walls of the worm-inhabited Wolfville and Windsor treatments. MeHg concentrations in the surface sediments were consistently lower than in the bulk samples. The percentage of MeHg in surface samples of worm-inhabited microcosms decreased in Wolfville (1.5 to 1.2 %) and Windsor (1.4 to 0.8 %) sediments compared to the worm-free treatments. However, %MeHg in burrow walls of worm-inhabited Wolfville (1.6 %) and Windsor (1.2 %) treatments were similar to surface sediments of control treatments. This research indicates that the worms reduced MeHg concentrations in the surface sediment, possibly due to feeding and bioaccumulation in their tissues. In addition mercury was demethylated in the burrow walls due to ventilation of burrows with aerated seawater and subsequent aeration of the surrounding sediment. This has important implications for mercury biogeochemical cycling in coastal ecosystems.

**32 Comparison of Mercury Localization, Speciation, and Histology in Multiple Fish Species from Caddo Lake, a Fresh Water Wetland** J. Smith, Univ of North Texas; M.M. Chumchal, Texas Christian Univ, Biology Dept; J. Schulmeier, Dermatopathology Associates; G. Verbeck, Univ of North Texas; B.W. Matherne, Univ of North Texas, Dept of Biological Sciences; B. Barst, Université du Québec, INRS-ETE; A. Roberts, Univ of North Texas. Mercury (Hg) contamination is a global problem with implications for both ecosystem and human health. Concentrations of Hg in Caddo Lake (TX/

LA, USA) fish are within the range of those reported by other authors to result in reduced fitness and reproduction. Measures of melanomacrophage aggregates (MA) in fish have been correlated with exposure to a variety of environmental contaminants including Hg and also vary in tissue of residence based on fish species. Mercury has been shown to accumulate in MA in gar from Caddo Lake. However, the mechanistic relationship between MA and Hg is unknown. Gar from Caddo Lake show very high proportions of inorganic mercury in the liver, a tissue that, in gar, has extensive MA. Bass from Caddo Lake show much lower levels of inorganic mercury in liver than gar, and have no discernable MA. This study examined the livers and spleens of wild fish from Caddo Lake to determine associations between MA content, mercury speciation, localization, and tissue histology. Liver, spleen, and muscle samples from spotted gar (*Lepisosteus oculatus*), largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*) and bowfin (*Amia calva*), were collected from wetland habitat types. Evidence was sought for liver and spleen pathology association with mercury concentration and speciation in liver and spleen. Laser ablation ICP-MS was used to compare Hg accumulation between MA and histologically unremarkable parenchyma cells in liver and spleen.

### 33 Modelling Mercury Biomagnification Through Acidic Lake Food

**Webs** M. Clayden, Canadian Rivers Institute, Univ of New Brunswick, Biology Dept; B. Wyn, North South Consulting; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; J. Kirk, D. Muir, Environment Canada, Water Science and Technology Directorate; N. O'Driscoll, Acadia Univ, Dept of Earth and Environmental Sciences. Kejimikujik National Park is a relatively remote and pristine area in southwestern Nova Scotia, Canada. Nonetheless, it receives mercury (Hg) and acidifying pollutants in precipitation due to its location downwind of industrial and urban centres in the northeastern United States and southern Canada. As such, the pH of lakes in the park has declined beyond naturally low levels, and Kejimikujik has been identified as a hotspot of biological Hg contamination in North America. Between 2006 and 2010, we conducted food web studies on 11 lakes in the park with a range of physical and chemical characteristics. Our aims were: 1) to compare Hg biomagnification in these lakes to near neutral systems; and 2) to determine whether chemical characteristics (e.g., pH, trophic status, metals) explained any among-lake differences in Hg biomagnification or its concentrations in lower trophic levels. To do this, we measured methyl Hg (MeHg) and total Hg (THg) in zooplankton, macroinvertebrates, fishes, sediments and water, as well as sources of energy (using S and C isotope ratios) and trophic position (using N isotope ratios) of the biota, and chemical characteristics of the lakes. As in other studies, we used the linear regression slope of log Hg versus N isotope ratios to model Hg biomagnification through the food web of each lake. Although our study lakes are acidic and oligotrophic (pH 4.6 – 6.0, total phosphorus (TP) 0.006 – 0.014 mg/L), regression slopes ranged from  $0.16 \pm 0.01$  to  $0.23 \pm 0.01$  ( $R^2 = 0.72 - 0.91$ ;  $p < 0.001$ ), similar to those from more neutral and eutrophic freshwater systems. We found that food web biomagnification of Hg was negatively correlated with trophic status across lakes (total nitrogen (TN),  $r = -0.72$ ,  $p = 0.018$ ; TP,  $r = -0.71$ ,  $p = 0.022$ ). Levels of Hg at the base of the food webs were also negatively related to TN ( $r = -0.66$ ,  $p = 0.039$ ) and pH ( $r = -0.81$ ,  $p = 0.005$ ), but positively related to dissolved organic carbon ( $r = 0.80$ ,  $p = 0.006$ ), and some metals (e.g., aluminium,  $r = 0.88$ ,  $p < 0.001$ ). Our results suggest that Hg biomagnification and its concentrations at the base of the food web and in fish are affected by chemical characteristics of the lakes. Further research will compare whether food web structure, including sources of energy, also influence Hg concentrations in the biota of these lakes.

### 35 Measurements and Model Results of Mercury Air-Sea Exchange Near Bermuda

**A.L. Soerensen**, Harvard Univ, Department of Environmental Health; E. Sunderland, Harvard Univ; R. Mason, M. Andersson, P. Balcom, Univ of Connecticut. New global modeling results show evasion of elemental Hg (Hg(0)) from the ocean is comparable in magnitude to direct anthropogenic emissions. Results show that 80% of the Hg load deposited to the ocean surface reenters the atmosphere as the result of air-sea exchange, while 20% will accumulate in the subsurface ocean. Efforts to understand air-sea exchange on a global scale are currently constrained by sparse measurement data. However, the possibility of simultaneous measurements of Hg(0) in air and water now makes it possible to calculate air-sea exchange with a high temporal resolution and to validate model simulations of concentrations and fluxes at the air-sea interface. Here we compare air-sea

exchange based on cruise observations and model simulations with a 3-hour average temporal resolution. Measurements of dissolved Hg(0) in seawater and total atmospheric gaseous mercury (TGHg) are from four cruises in the West Atlantic Ocean between 2008–2010. Seawater Hg(0) was estimated from equilibrium air concentrations determined every 5 minutes with a Tekran 2537A and collected using a continuous sparging set-up with a high water/air flow ratio that ensured gas:water equilibrium (seawater flow was 10–16 L/min and the Hg air flow 1.5 L/min). Water concentrations were calculated from these measured air concentrations using Henry's law. Atmospheric concentrations were measured every 5 minutes using another Tekran 2537A. Results show average atmospheric cruise concentrations of 1.4–2.3 ng/m<sup>3</sup>. Measured average seawater Hg(0) concentrations ranged from 0.06–0.14 pM during the cruises, while the corresponding fluxes of Hg(0) to the atmosphere predicted from the observational data were between 10–24 pmol/m<sup>2</sup>/hr, depending on wind speed and water temperature. Comparison of these observations to modeled results from the surface-slab ocean simulation for Hg in the GEOS-Chem biogeochemical model shows reasonable agreement. The model underestimates seawater concentrations in coastal regions and during a strong summer storm. We discuss recent improvements to the model to improve overall performance and present sensitivity results from a various air-sea exchange parameterizations.

### 36 The Presence and Measurement of Organic Mercury Species in the Arctic Troposphere

**P.A. Baya**, H. Hintelmann, Trent Univ, Environmental and Life Sciences. Mercury (Hg) is a persistent atmospheric pollutant that exhibits long range transport and bio-accumulates in the aquatic ecosystem in the form of monomethylmercury (MMHg), the toxic form of mercury. The sources and fate of MMHg in the ecosystem and the contribution of the atmosphere is however still unclear. Even if the atmosphere is the major pathway of Hg contamination in the arctic, the direct and indirect atmospheric MMHg contribution to the aquatic ecosystem is not well understood due to the lack of known reliable measurement methods for organic Hg in the atmosphere. In the arctic open waters where there is net deposition of Hg, DMHg can be volatilized from surface water as suggested in various studies. The atmospheric DMHg may quickly degrade to MMHg and is then potentially deposited to snow packs on ice fields or back to open waters. Such a behavior would make DMHg a major source of MMHg to the arctic ecosystems. To establish the presence of organic Hg species in arctic troposphere, an analytical method was developed. The method is based on species specific Hg isotopic dilution and online ethylation of MMHg from air samples and trapping of ethylated MMHg and DMHg on Tenax traps. This study presents for the first time concentrations of organic mercury species (monomethylmercury and dimethylmercury) in the arctic lower atmosphere (Hudson Bay and Canadian Archipelago) measured during the CGCS Amundsen expedition in summer 2010. The Hudson Bay airshed is dominated by MMHg ranging from 3.9 – 8.1 (mean =  $5.5 \pm 2.0$ ,  $n=5$ ) pg/m<sup>3</sup>, while concentrations of DMHg ranged from < LOD to 1.6 (mean =  $0.8 \pm 0.6$ ,  $n=4$ ) pg/m<sup>3</sup>. In the high Arctic however, DMHg concentrations are highest ranging from 1.8 to 9.6 (mean =  $4.3 \pm 2.1$ ,  $n=12$ ) pg/m<sup>3</sup>. MMHg levels were significantly lower than those measured in Hudson Bay ranging from < LOD to 5.2 (mean =  $1.7 \pm 1.7$ ,  $n=12$ ) pg/m<sup>3</sup>. These findings support the hypothesis that organic mercury species can be volatilized from the ocean and contribute directly to MMHg bio-accumulation in the arctic ecosystem. The concentrations of organic Hg in water as well as the extent of DMHg photodegradation may be important factors affecting the organic Hg species concentrations. The potential sources and factors affecting organic Hg species concentrations and compositions in arctic air will be further discussed in the presentation.

### 37 Methylmercury Cycling in High Arctic Wetlands: What Are the Controls on MeHg Production?

**I. Lehnher**, Univ of Waterloo, Earth and Environmental Sciences; V.L. St Louis, Univ of Alberta, Biological Sciences; J.L. Kirk, Research Scientist, Environment Canada; C.A. Emmerton, Univ of Alberta, Biological Sciences; J.D. Barker, Ohio State Univ. Some freshwater fish in the Arctic contain levels of methylmercury (MeHg) that pose health risks to Inuit peoples harvesting these species as traditional food. In temperate regions, wetlands are known sources of MeHg; however, the importance of wetlands to Hg methylation in the Arctic is unclear and the sources of MeHg to arctic freshwater ecosystems are still unidentified. The objective of this study was to quantify MeHg production in wetland ponds near Lake Hazen (81°N) in the Canadian Arctic and determine the biogeochemical controls on MeHg production. To quantify net in-pond



production of MeHg during the summer seasons of 2005, 2007 and 2008, mass-balance budgets were constructed for two wetland ponds (Ponds 1 and 2) by measuring external MeHg inputs from atmospheric deposition, MeHg losses from photodemethylation, and changes in MeHg storage in the water column. MeHg inputs from precipitation were small (0.026–0.051 ng/m<sup>2</sup>/d). Photodemethylation was a sink for 77–122% of MeHg produced in-pond, and was controlled by the attenuation of UV-A radiation in the water column. In-pond MeHg production ranged from 14–40 ng/m<sup>2</sup>/d in Pond 1 and 1.7–1.9 ng/m<sup>2</sup>/d in Pond 2, comparable to what has been reported for temperate lakes and wetlands. Flooding of the Pond 1 wetland in 2008 due to rising water levels in nearby Lake Hazen resulted in increased MeHg production. Additionally, potential rates of Hg(II) methylation were quantified in intact sediment cores (n=29) from a number of ponds using Hg stable-isotope tracers to identify the factors controlling MeHg production. Potential methylation rates were high and exhibited nearly as much intra-site as inter-site variability. Sediment MeHg concentrations were significantly correlated to methylation potential and sediment total-Hg concentrations. Furthermore, pond water MeHg concentrations were positively correlated with sediment MeHg concentrations, indicators of anaerobic microbial decomposition of organic matter ( $pCH_4$ ,  $NH_4^+/NO_3^-$  ratio), and negatively correlated with UV-A exposure and particulate carbon. However, no correlation with sulfate was observed. Our results demonstrate that Arctic wetland sediments are sources of MeHg comparable to freshwater sediments in temperate latitudes and that MeHg concentrations in water are controlled by production in sediments – itself a function of anaerobic microbial activity, methylation potential and Hg(II) availability – as well as photodemethylation in the water column.

**38 In Situ Optical Measurements as Proxies for Stream Mercury** L. Shanley, G. Aiken, B. Pellerin, J. Saraceno, US Geological Survey; A. Riscassi, Univ of Virginia; C. Driscoll, Syracuse Univ; B. Beramaschi, US Geological Survey. Recent advancements in inexpensive in situ optical sensors permit continuous measurements that reveal information about the quantity and quality of stream organic matter. Due to the close relation between organic matter and mercury transport, we have applied in situ fluorescing dissolved organic matter (FDOM) as a proxy to generate continuous time series of aqueous mercury (Hg) concentrations. Typically only about 2% of organic matter fluoresces, but the measurement is highly sensitive and fairly specific to the fraction of DOM that is associated with Hg transport. We have measured FDOM in three northeastern USA forested research catchments and compared in situ FDOM to total dissolved Hg concentrations in discrete samples. Correlations of dissolved total Hg with in situ FDOM were somewhat lower than anticipated due to occasional interference of FDOM by high turbidity. However, with accurate and continuous turbidity measurements, one can compensate for this source of error. Optical turbidity measurements have the added advantage of serving as a proxy for particulate organic matter, which is often highly correlated to particulate Hg, the dominant form of Hg export in some streams. By combining these proxies, it is possible to quantify the majority of Hg flux in these streams. Because dissolved and particulate Hg both vary strongly but not always predictably with flow, these high frequency optical proxies greatly improve the accuracy of Hg flux calculations.

**39 Molecular Mechanisms of Interest in Nanomaterial Toxicity in Aquatic Species: Beyond Oxidative Stress** R. Klaper, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences, Great Lakes Water Institute; D. Arndt, Univ of Wisconsin-Milwaukee, Great Lakes Water Institute, School of Freshwater Sciences; J. Crago, Univ of Wisconsin-Milwaukee, Great Lakes Water Institute; F. Goetz, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences; J. Chen, Univ of Wisconsin-Milwaukee, Chemistry. Much of the focus of nanotoxicology to date has been on the impact of various particle types on acute mortality in various model systems. Mechanistic information on how a nanomaterial may cause toxicity has had a significant focus on oxidative stress. However, oxidative stress assays provide limited information regarding how an organism may interact with a particle type and how nanomaterials may differ in their interactions with organisms. Different molecular mechanisms may be triggered depending on nanomaterial chemistry. This presentation will discuss how nanomaterials with differing structure and surface chemistry differ in their impact on the molecular profiles of the model organisms *Daphnia* and rainbow trout. Several carbon-based nanomaterials were used in exposures including fullerenes (nC<sub>60</sub>) and single-walled carbon nanotubes as well as fullerenes modified

with hydroxylation and  $\beta$ -Cyclodextran, and single walled carbon nanotubes modified with PEG, carboxylic acid and NH<sub>2</sub>. We have also tested the metal oxide titanium dioxide. Our research has shown that the environmental impact of manufactured nanomaterials clearly differs with nanomaterial and concentration and the mechanisms triggered by each nanomaterial also differs. In *Daphnia*, genomic expression data were gathered using high-throughput sequencing and monitored across particle types and exposure concentrations and durations using QPCR of individual genes to determine potential mechanisms that may be acting across different exposure types. Nanomaterial exposure causes the up or down regulation of genes associated with several important pathways that include oxidative stress but other important functions as well such as immune response, growth and molting, protein synthesis, reproduction and others. In rainbow trout microarrays of important immune genes indicate differences in the number of genes and expression profiles across particle types. In both studies we have found that individual gene expression and global patterns differ with nanomaterial functionalization or surface chemistry indicating that surface chemistry may be more important than just core chemistry in determining molecular responses of aquatic species to nanomaterials.

**40 The Effects of Metal Nanoparticles on ROS Production and Developmental Processes in Marine Organisms** S. Gilbert, Univ of North Carolina at Charlotte, Dept of Biology; M. McCarthy, Univ of North Carolina at Charlotte; L. Marston, North Carolina State Univ; D. Carroll, Wake Forest Univ, Center for Nanotechnology and Molecular Materials; N. Levi-Polyachenko, Wake Forest Univ, Dept of Plastic and Reconstructive Surgery; A. Ringwood, Univ of North Carolina at Charlotte. The importance of evaluating the biological effects of engineered nanoparticles on marine invertebrates is becoming increasingly evident. The manufacture and use of nanoparticles continues to rise and the probability of these nanoparticles entering the aquatic ecosystem is increasing. Thus, the purpose of these ongoing studies is to characterize the toxicity of various metal nanoparticle preparations on developmental forms of oysters, *Crassostrea virginica* and sea urchins, *Arbacia punctulata*. Studies are being conducted with embryos and larvae of both species to examine and compare the effects of metal nanoparticles on protostomic and deuterostomic development. Newly fertilized oyster and sea urchin embryos were exposed to three types of silver nanoparticles (seeds, prisms, and plates) over a range of concentrations, and then the percent normal development as well as reactive oxygen species (ROS) levels were assessed. Older sea urchin and oyster larvae were also exposed to a range of silver nanoparticle concentrations for 7 days and growth was assessed. Silver nanoparticles resulted in a change in ROS production in embryos, and there was evidence of shape dependent differences in toxicity. Similar studies were also conducted using TiO<sub>2</sub> nanoparticles. These basic studies are essential for addressing the potential impacts of engineered nanoparticles on fundamental developmental and cellular processes.

**41 Phototoxicity of TiO<sub>2</sub> Nanoparticles Under Solar Radiation is Dependent on Solar UV Spectrum** H. Ma, A. Brennan, US Environmental Protection Agency, Mid-Continent Ecology Division; S. Diamond, US Environmental Protection Agency, MED. Titanium dioxide nanoparticles (nano-TiO<sub>2</sub>) are currently used in a broad range of applications from photocatalysts to personal-care products. Phototoxicity of these nanoparticles has become an important concern as some forms can be readily photoactivated under environmental UV radiation (i.e., solar radiation) and generate reactive oxygen species (ROS). In this study, photocatalytic ROS production and phototoxicity of nano-TiO<sub>2</sub> (Aeroxide P25) to *Daphnia magna* were investigated under different solar UV spectrum by applying a series of spectral filters (i.e., standard window glass, microscope slide, petridish glass, acrylic glass, 345 nm, 360 nm, and 400 nm cutoff filters) within a solar simulator. Photocatalytic ROS production was measured by APF [3' (*p*-aminophenyl) fluorescein] assay, a fluorescent-based ROS assay with great specificity to OH•. Phototoxicity was evaluated by immobilization of *D. magna*. Intracellular ROS production was determined by H<sub>2</sub>DCFDA assay. Microscope slide, petridish glass, and standard window glass, which gradually block UVB (280–320 nm), had no impact on photocatalytic ROS production by nano-TiO<sub>2</sub>; whereas the 345 nm and 360 nm cutoff filters, and acrylic glass (with an approximate cutoff at 380 nm), which gradually block UVA (320–400 nm) from solar radiation, decreased ROS production by 8%, 31%, and 94%, respectively, as compared to no filter, at 1000 ppb TiO<sub>2</sub>. The 400 nm cutoff filter decreased ROS production by 100%, proving the band gap theory concept for TiO<sub>2</sub> nanoparticles, that only wavelength at



or below 388 nm can induce photoactivation. The impact of these spectral filters on phototoxicity of nano-TiO<sub>2</sub> was well in agreement with their impact on photocatalytic ROS generation of the nanoparticles. Standardized to control (no filter treatment), immobilization of *D. magna* by 500 ppb nano-TiO<sub>2</sub> under different filters showed the following order: no filter (100%) > standard window glass (92%) > 345 nm cutoff (57%) > 360 nm cutoff (15%) > acrylic glass (4%) > 400 nm cutoff (0%). A linear correlation was found between ROS production of nano-TiO<sub>2</sub> and its phototoxicity to *D. magna* ( $r=0.97$ ,  $n=5$ ,  $p<0.001$ ), suggesting that photocatalytic ROS production may be a predictor of phototoxicity for these nanoparticles. Intracellular ROS production was consistent with *D. magna* immobilization, indicating oxidative stress was involved in the phototoxicity. Findings from this study demonstrate that phototoxicity of nano-TiO<sub>2</sub> can occur under natural solar radiation and variation of solar radiation spectrum may have significant impact on phototoxicity of these nanoparticles.

**42 Nanoparticle Uptake in Freshwater Aquatic Macrophytes Is Size and Species Dependent** B. Glenn, Clemson Institute of Environmental Toxicology; S.A. White, Clemson Univ, Clemson Institute of Environmental Horticulture; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX). Partitioning of 4nm and 18nm spherical gold nanoparticles (AuNPs) to aquatic macrophytes was investigated in vivo. Three morphologically distinct macrophytes were studied. *Myriophyllum simulans* Orch. and *Egeria densa* Planch. are submerged aquatic vascular plants while *Azolla caroliniana* Willd. is a free-floating aquatic fern. Since aquatic plants absorb the majority of their nutrients from the water column; it is logical to hypothesize that they may absorb nanomaterials in suspension. Each plant was exposed to nominal concentrations of 250µg/L AuNPs for 24hrs. Macrophytes were harvested at 6 time-points (1,3,6,12,18 and 24hrs), dried and then analyzed for gold concentration via inductively coupled plasma mass spectrometry. Concentrations were normalized to whole plant dry tissue mass. Here we show absorption of AuNPs via root uptake was size and species dependent. Electron microscopy revealed that 4nm and 18nm AuNPs adsorbed to the roots of each species. Root tissue was sectioned and transmission electron microscopy indicated that 4nm and 18nm AuNPs were absorbed by *A. caroliniana*, however only 4nm AuNPs were absorbed by *M. simulans*. *Egeria densa* did not absorb AuNPs of either size. AuNPs were confirmed in tissue using energy-dispersive X-ray spectroscopy. AuNP absorption by plants may be a function of salinity tolerance of each species.

**43 Disruption of Intestinal Microbiota in Fish After Ingestion of Some NPs Indicates Potential for Effects of Dietary NP Exposure on Organism Health** T. Henry, Univ of Plymouth, School of Biomedical and Biological Sciences, School of Biology; D. Merrifield, Univ of Plymouth, School of Biomedical and Biological Sciences. Nanoparticles are used in some food and food packaging applications and ingestion NPs can occur in organisms. In addition, food consumed by organisms can be contaminated by NPs if NPs are released into the environment. Ingested NPs could cause toxicity if they are absorbed and transported within organisms to vulnerable tissues and the issue of whether NPs are absorbed across epithelial membranes is a significant focus within the emerging discipline of nanotoxicology. However, it is possible that negative effects of NPs can occur in organisms without absorption of the particles across epithelial membranes. We evaluated whether ingested NPs [Cu-NPs, Ag-NPs, C<sub>60</sub>, and single walled carbon nanotubes (CNTs)] incorporated into food pellets could affect the intestinal epithelium or microbial community structure within the gut of adult zebrafish. Zebrafish food (pellets) were prepared containing NPs (500 mg/kg food), and control food supplemented with either CuSO<sub>4</sub> or AgNO<sub>3</sub> (equivalent metal concentrations) or carbon black (equivalent carbon concentrations), and a control food without added metal or carbon. Adult zebrafish were fed for 14 d and intestines were sampled for assessment of microbial community: bacterial DNA extraction, PCR amplification of the 16S rRNA V3 region, separation of PCR products by DGGE analysis, and sequence analysis. Presence of lesions and absorption of NPs across intestinal epithelia were assessed by EM. No treatment effects on food palatability, feeding rate, fish behavior or mortality occurred; and no evidence of NP absorption was observed. Changes in intestinal microbial community structure were minimal for all Ag treatments relative to the control (similarity coefficients >91%), but Cu treatments caused substantial alteration of community structure (< 65% and < 43% similarity relative to control, for Cu and Cu-NP treatments respectively). For the metal NPs, Cu-NP treatment had greatest effect on

microbial community structure and inhibited some endogenous and beneficial bacterial strains to non-detectable levels and was clearly more disruptive than elemental Cu exposure. Analysis of effects of carbon NPs on microbiota and intestinal epithelia are ongoing. The potential for some types of NPs to change intestinal microbial community structure could affect nutritional status, ability to defend against pathogens, and overall organism health.

**44 Interactions of C<sub>60</sub>, Multi-Wall Carbon Nanotubes (MWCNT), and Single Wall Carbon Nanotubes (SWCNT) with Natural Organic Matter, Bacteria and Biofilms** J.R. Lawrence, M.J. Waiser, Water Science and Technology Directorate, Environment Canada; G. Swerhone, Water Sciences and Technology Directorate, Environment Canada; V. Tumber, Water Science and Technology Directorate, Environment Canada; J. Roy, Water Science and Technology Directorate, Environment Canada; A. Paule, Université de Toulouse, EcoLab Laboratoire d'écologie fonctionnelle; J.J. Dynes, Canadian Light Source Inc; D.R. Korber, Univ of Saskatchewan, Food and Bioproducts Sciences. The commercial production of nanoparticles (NP) (those with at least one dimension < 100 nm) has generated a need for research to support regulation of the nanotechnology sector. In the current study, scanning transmission x-ray microscopy (STXM) at the C 1s edge was used to study the fate of a variety of carbon nanomaterials in a complex natural river biofilm, demonstrating rapid aggregation and extensive coating with lipo-protein and carbonates. Although modified by sorption processes, these carbon-based materials were shown, using confocal scanning laser microscopy, conventional and molecular analyses, to have significant effects on community structure and function. Microbial biofilm communities were developed in rotating annular reactors during continuous exposure to 500 µg L<sup>-1</sup> of each nanomaterial. Microscopy observations indicated that the communities were visibly different in appearance with changes in abundance of filamentous cyanobacteria, in particular. Microscale analyses indicated that fullerene did not significantly impact algal, cyanobacterial or bacterial biomass. In contrast, MWCNT exposure resulted in a significant decline in algal and bacteria biomass. Interestingly, the presence of SWCNT products significantly increased algal biomass but had no significant impact on cyanobacterial or bacterial biomass. Thymidine incorporation indicated that bacterial production was significantly reduced by all nanomaterials with the exception of fullerene. Biolog assessment of community carbon utilization patterns revealed few significant effects. PCA and ANOSIM analyses of denaturing gradient gel electrophoresis (DGGE) results indicated that the bacterial communities exposed to fullerene were not different from the control, the MWCNT and SWNT-OH differed from the control but not each other, whereas the SWCNT and SWCNT-COOH both differed from all other treatments and were significantly different from the controls. Fluorescent lectin binding analyses also revealed changes in microbial community structure during exposure to all tested nanoproducts. Observations indicated that at 500 µg L<sup>-1</sup>, carbon nanomaterials significantly altered microbial community structure and function, supporting the need for further evaluation of their effects in aquatic habitats.

**45 Effects of Silver Nanoparticles on Filter-feeding Tadpoles in Different Source Waters** C.M. Fridgen, M. Xenopoulos, Trent Univ, Biology; C. Metcalfe, Trent Univ, Environmental and Resource Studies. Engineered silver nanomaterials (nAg) are widely used in a variety of products and have the potential to be released into the aquatic environment. Recent research has shown variable toxicity of nAg to aquatic organisms, which could be due to a range of factors, including the release of silver ion, the capping agent and the chemical composition of the water. In this study, we tested for the acute toxicity of a carboxy-functionalized capped nAg with a reported particle size of 2–10 nm sold by ViveNano (Toronto, ON, Canada) and AgNO<sub>3</sub> on the common bull frog tadpole (*Rana catesbeiana*) reared in water from seven different sources (2 rivers, 2 natural ponds, 2 urban storm water ponds) and dechlorinated tap water. LC<sub>50</sub> (96 h) values for the nAg varied significantly between water sources, ranging from 141±36 µg/L (tap water) to 474±21 µg/L (pond water). In all source waters, AgNO<sub>3</sub> at 100 µg Ag/L caused greater mortalities to the tadpoles than nAg at 200 µg Ag/L. However, in one source water (i.e., natural pond) with especially high dissolved organic carbon (DOC), AgNO<sub>3</sub> was not toxic. Out of all water parameters assessed (ph, common ions and nutrients) DOC was the only factor which showed correlation with the fate and toxicity of Ag. For treatments with AgNO<sub>3</sub>, tadpole mortality was significantly reduced with increasing DOC ( $r=-0.94$ ,  $p=0.001$ ), but DOC had no significant effect on nAg toxicity. Using ICP-OES, we measured total Ag in the water column and tadpoles

at 0 and 96-hrs of exposure to nAg and AgNO<sub>3</sub>. The amount of total silver that persisted in the water over 96 hrs was positively correlated with DOC ( $r=0.71$ ,  $p=0.008$ ) in the AgNO<sub>3</sub> treatments, but persistence was not related to DOC in the nAg treatments. Whole-body Ag content in the tadpoles was linearly correlated with mortality rate in the AgNO<sub>3</sub> treatment ( $r=0.72$ ,  $p=0.012$ ) but not in the nAg treatments. These data indicate that nAg is not as acutely toxic as AgNO<sub>3</sub> to the tadpoles and that DOC has little effect on the toxicity of this carboxy-functionalized material. While the free silver ion may be a toxic component of nanosilver products, trends for toxicity and fate of a carboxylated nano silver products did not follow the same trends as was observed with AgNO<sub>3</sub>. More research will be conducted looking at chronic exposure of the carboxylated silver nanoparticles in various exposure waters.

**46 Silver Nanoparticle Toxicity to Atlantic Killifish and Zebrafish in Complex Environmental Media: Laboratory, Mesocosm and Microcosm Studies** A.J. Bone, Duke Univ, Nicholas School of the Environment; C.W. Matson, Duke Univ, CEINT, Baylor Univ, Environmental Science; B. Colman, Duke Univ, CEINT; R.T. DiGiulio, Duke Univ, Nicholas School of the Environment, CEINT. The increasing use of silver nanoparticles in consumer products and industrial applications has created concern over their inevitable release into the aquatic ecosystem. In this study, the effect of complex environmental media on silver nanoparticle toxicity was investigated using gum arabic coated silver nanoparticles (Ag-GA NP), polyvinylpyrrolidone coated silver nanoparticles (Ag-PVP NP), silver nitrate (AgNO<sub>3</sub>) treated mesocosms containing sediment and aquatic plants modeled after North Carolina wetlands. A follow up microcosm experiment was used to investigate the role of different sources of dissolved organic material (DOM) on silver nanoparticle toxicity. Surface water samples were taken from the mesocosms at 24 hours after dosing and compared to lab-incubated samples prepared by spiking water taken from untreated control mesocosms with Ag-GA NP, Ag-PVP NP and AgNO<sub>3</sub> in the lab at the same concentrations observed in the mesocosms at 24 hours. Mesocosm samples and lab-incubated samples were compared using acute toxicity testing for mortality to early life stage Atlantic killifish (*Fundulus heteroclitus*) and zebrafish (*Danio rerio*). Any differences in the toxicity between the lab-incubated water and the mesocosm water could thus be attributed to the more complex environment created by introducing plants, sediment and general environmental conditions such as sunlight in addition to the natural water chemistry. In general, lab-incubated samples were more toxic than samples taken from the mesocosms. However, for Ag-PVP NP, mesocosm samples were more toxic than lab-incubated samples, indicating that an environmental factor unique to the mesocosms is causing increased toxicity of Ag-PVP NP. In addition, for mesocosm samples, both Ag-GA NP and Ag-PVP NP were more toxic than AgNO<sub>3</sub> on an equal mass basis. Therefore, the complex media is acting differently on Ag<sup>+</sup> to reduce its toxicity in a manner that does not translate to Ag-GA or Ag-PVP NP. Microcosm experiments using the same particles with four environmental scenarios (water only, sediment + water, plants + water, and plants+sediment+water) indicate that plants are the strongest protective factor and that sediment may have contributed to the increased toxicity of Ag-PVP NP. These results suggest that conventional laboratory testing and equation of silver nanoparticles to ionic silver on a mass basis may be inadequate for silver nanoparticle risk assessment.

**47 Integration of Density Dependence and Concentration Response Models Provides an Ecologically Relevant Assessment of Populations Exposed to Toxicants** S. Raimondo, US Environmental Protection Agency, Gulf Ecology Division, National Health and Environmental Effects Laboratory; H. Rutter, Student Services Contractor to USEPA; C.R. Jackson, B. Hemmer, US Environmental Protection Agency. The assessment of toxic exposure on wildlife populations involves the integration of organism level effects measured in toxicity tests (e.g., chronic life cycle) and population models. These modeling exercises typically ignore density dependence, primarily because information on density dependence functional forms is lacking for most species. The interactions between population density and toxicant exposure can be synergist or antagonistic, resulting in either under or overestimation of toxicant effects based on low density toxicity tests and density independent models. To explore the relationship between population density dependence and toxicant concentration, we simulated the exposure of a density dependent sheepshead minnow (*Cyprinodon variegatus*) population to five chemicals of various modes of action (estradiol, trenbolone, trifluralin, chlordane, pentachlorophenol). The functional form of density

dependence was derived from laboratory density manipulation experiments that modeled the influence of adult density on juvenile survival and growth and adult survival and reproduction. Concentration-response models were developed from the chronic toxicity tests for all endpoints within each chemical where significant effects were measured. For each chemical, exposure was simulated across a wide range of densities and concentrations, applying density dependent mechanisms and concentration response curves at each density-concentration combination. The relationship between density and toxicant concentration for each chemical was modeled and described using population growth rate (PGR) contours. These contours identify compensatory and synergistic responses over a range of densities and concentrations for each chemical and demonstrate alterations to "carrying capacity" (PGR = 0) relative to stress and density, which provides the boundary of a population's tolerance to the model stressor. This work demonstrates how the integration of intraspecific interactions and organism toxicant response can provide a more ecologically relevant assessment of population response to toxicant exposure.

**48 A Framework for Integrated Fish Monitoring: An Example Based on 20 Years of Data for Perch** N. Hanson, Univ of Gothenburg, Dept of Plant and Environmental Sciences; L. Forlin, Univ of Gothenburg, Dept of Zoology; A. Larsson, Univ of Gothenburg, Dept of Plant and Environmental Sciences. Environmental monitoring is performed to provide information that can be used by environmental managers to protect ecological resources. Chemical and physical measurements have for a long time been used as the primary source of information. However, such measurements are not helpful when environmental degradation is caused by unexpected chemicals or processes. Biological measurements can be used to get an integrated picture of all stress that affects the ecosystem. Sub-organismal biomarkers provide information about the health and exposure of individuals, and can be used as early warning signals for new or unexpected chemicals. Biomarkers may also give some information about the cause of the stress. However, the ecological services that we want to protect are performed by populations. Therefore, effects on the population level may have higher relevance for management. The population level integrates everything that affects the population, including non chemical stress. A drawback with the population level is that it is difficult to establish causal relationships to identify the stressor. Fish are used frequently for environmental monitoring. Different types of strategies for fish monitoring include chemical measurements in fish tissue, biomarker responses, and the size and structure of fish populations and communities. All these parts are included in the program for Integrated Fish Monitoring, which is a part of the Swedish Environmental Monitoring Program. This program was started to provide reference data for investigations of potentially contaminated sites. However, the reference data has not been used to the extent that was intended. One of the reasons for this is that there is no guidance on how to integrate the responses of the different measurements in a way that is informative for environmental managers. Based on the program for Integrated Fish Monitoring, we have developed a framework for integrating responses from five lines of evidence (LOE); contamination, eutrophication, biomarkers of exposure, fish physiology, and the population level. Each of the five LOE provides important information about the health of the aquatic ecosystem. Based on the available data, each of the five LOEs is determined to be either affected or not affected, resulting in 32 possible outcomes. To aid risk assessors and environmental managers, a decision matrix was set up with interpretations and recommended action for each of the 32 possible outcomes.

**49 Endocrine Disruption in an Urban Estuary and its Catchment Drains** D. Webb, Curtin Univ, Environment and Agriculture. The Swan-Canning Estuary (SCE) is of environmental and cultural importance to the population of Perth, Western Australia. Like all urban estuaries it has become increasingly eutrophic marked by annual algal blooms due to over or incorrect application of fertilisers. Fish, and recent dolphins, deaths have highlighted other water quality issues potentially due to non-nutrient contamination. Furthermore, there has been a decline in the populations of several fish species such as cobbler and Perth herring as well as the almost disappearance of the western school prawn (*Metapenaeus dalli*) from the estuary. A pilot study in catchment drains discharging to the SCE show evidence of endocrine disruption and impaired DNA repair pathways in freshwater shrimp (*Palaemonetes australis*). There was a strong bias towards females in all drains, and intersex was above the normal incidence in crustaceans at several sites. Biomarker and intersex results recorded in this study

have implications for the long term health of all crustacean species within the influence of the types of contaminants measured in the estuary and its tributary drains. Studies using male *Gambusia holbrooki* have identified fish with impaired gonopodial development adding to the evidence of the presence of endocrine disrupting chemicals in drainage to the SCE. Because of the scope of global climate change further stresses will be added to communities already impacted by anthropogenic disturbances. Investigations are planned to study both direct and indirect biological effects to aquatic biota due to changes predicted to occur in the SCE and its surrounding catchment as a result of declining stream flows.

**50 Mercury Bioaccumulation in Elasmobranchs** N.J. Kutil, Roger Williams Univ, Marine Biology; M. Soto, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología. Mercury (Hg) is a toxic environmental contaminant that bioaccumulates in fish tissues, including numerous marine species. Cartilaginous fish of the subclass Elasmobranchii are important ecological constituents of marine ecosystems, yet the fate of Hg contaminants in their body tissues is largely unknown. In this study, four species of elasmobranchs: little skate (*Raja erinacea*), winter skate (*R. ocellata*), smooth dogfish (*Mustelus canis*), and spiny dogfish, (*Squalus acanthias*) were collected from the Rhode Island/Block Island Sound, and the Hg content (ppm wet wt) of white muscle tissue was analyzed using automated combustion atomic absorption spectrometry. Diet and feeding habits for each species were also assessed by stomach content and stable nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope analyses. Mean Hg concentrations differed significantly among species, with highest levels measured in smooth dogfish (mean Hg =  $0.768 \pm 0.154$  ppm,  $n = 10$ ), followed by spiny dogfish (mean Hg =  $0.324 \pm 0.049$  ppm,  $n = 23$ ) and skates (mean Hg =  $0.100 \pm 0.012$  ppm,  $n = 36$  and  $0.064 \pm 0.005$  ppm,  $n = 23$  for little and winter skate, respectively). The Hg concentration of skate muscle tissue did not vary by body weight, suggesting that Hg does not bioaccumulate in these species. Conversely, smooth and spiny dogfish both bioaccumulate Hg with respect to body size, although smooth dogfish have a higher Hg content relative to spiny dogfish. The elevated Hg concentration of smooth dogfish may be explained by their higher trophic level status, as determined from  $\delta^{15}\text{N}$  signatures (mean  $\delta^{15}\text{N} = 13.55 \pm 0.81$ ,  $11.93 \pm 0.58$ ,  $12.43 \pm 0.59$ , and  $12.71 \pm 0.83$  for smooth dogfish, spiny dogfish, little skate, and winter skate, respectively). The enriched  $\delta^{13}\text{C}$  values of skates and smooth dogfish indicated benthic foraging (range of mean  $\delta^{13}\text{C} = -16.39 \pm 0.35$  to  $-17.69 \pm 0.42$ ), which was further confirmed by the dominance of decapod crustaceans in the stomach contents. Conversely, squid and butterfish were the principal prey of spiny dogfish, and the contribution of these pelagic prey was reflected in the depleted  $\delta^{13}\text{C}$  signature (mean  $\delta^{13}\text{C} = -21.98 \pm 0.82$ ). In future work, the effect of habitat use and Hg concentrations of principal prey will be examined to better understand bioaccumulation patterns among the elasmobranch species.

**51 Exposure of Cutthroat Trout to Carbaryl Following Application to Control Burrowing Shrimp – The Rest of the Story** C. Grue, Washington Cooperative Fish and Wildlife Research Unit, Univ of Washington, School of Aquatic and Fishery Sciences; J. Grassley, C. Elfes, A. Troiano, Univ of Washington. Labenia et al. (2007: Marine Ecology Progress Series 329:1-11) suggest the carbaryl exposures they used in laboratory studies with cutthroat trout (*Oncorhynchus clarki clarki*) and resultant adverse effects are likely to occur in Willapa Bay and Grays Harbor, Washington, following application of the insecticide to control burrowing shrimp thereby threatening local cutthroat trout populations. We captured cutthroat trout within channels adjacent to oyster beds before and after operational applications of the insecticide (8.97 kg of carbaryl [ai]/ha). Trout were captured during daylight high and low tides using gill nets and beach seines, respectively at -48, -24, 6, 24, 30, 54 and 72 h following applications in July 2007 and 2009. Fish were placed in wet ice in the field and frozen (-40 C) prior to brain acetylcholinesterase (AChE) assays. Maximum average enzyme inhibition in 2007 was 16% ( $n=2$  fish) compared to controls ( $n=7$ ) and occurred at 30 h post application; enzyme activity at other time points post spray was less than + 10% ( $n=11$ ). Similarly, in 2009 in which two applications separated by 3 days were monitored, maximum inhibition was 18% ( $n=2$ ) compared to controls ( $n=23$ ) at 24 h after each of the two applications. Enzyme activity at other time points post spray was also less than + 10% ( $n=50$ ). Results were similar to those reported previously for juvenile Chinook with maximum inhibition of 10% compared to controls. AChE inhibition in both species was significantly lower than that likely associated with the reductions

in swimming endurance and predator avoidance observed by Labenia and her co-workers. We conclude cutthroat trout are unlikely to be exposed to the concentrations and durations of exposure used by the authors in their experiments, and given spatial and ecological contexts, indirect effects of sufficient magnitude to adversely affect cutthroat trout populations in the Bay are unlikely.

**52 Vulnerabilities of Atlantic Sturgeon and Shortnose Sturgeon to PCB126 and TCDD Induced Early Life-stage Toxicities** I. Wirgin, New York Univ School of Medicine, Dept of Environmental Medicine; N.K. Roy, New York Univ School of Medicine, Environmental Medicine; R.C. Chambers, NOAA, Northeast Fisheries Science Center. Abundances of most sturgeon species worldwide are severely depleted. Overharvest, habitat alteration, and pollution are often cited as the three primary reasons for this decline, however, empirical demonstration of the effects of chemical toxicants are lacking. Shortnose sturgeon was federally listed as endangered in 1973 under the US Endangered Species Act and most populations of Atlantic sturgeon are currently under consideration for a similar designation. Populations of both species from several Atlantic coast estuaries are exposed to high levels of PCBs and PCDDs including those from the Hudson River PCBs Superfund site, however the vulnerabilities of their early life-stages to these AHR agonists have yet to be empirically evaluated. Initially, we partially characterized the cytochrome P4501A (CYP1A) gene and measured its expression in larvae of both species exposed under controlled conditions to graded doses of PCB126 and TCDD. We found that CYP1A expression was significantly inducible in both species at nominal water exposure concentrations as low as 10 ppt and 1 ppt, respectively. We also analyzed survivorship, hatching success and rate, and morphological alterations from these exposures in both species. Survival to hatching declined but days to hatching of survivors was accelerated at the highest doses of TCDD and PCB126. A syndrome of morphological toxic responses was also observed including shorter fish, yolk-sac edema, and retarded eye development at higher contaminant concentrations. However, post-hatching lifespan on yolk reserves alone (i.e., a food-free environment) was not affected by dose of toxicant. Our studies demonstrate that young life-stages of both species are dose sensitive to these two AHR agonists at environmentally relevant concentrations.

**53 Identification of Genes Related to Copper Metabolism in the South American Cyprinodontiform Fish *Poecilia vivipara*** E.S. Silva, J. Zanette, A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. South American cyprinodontiform fish like *Poecilia vivipara* are potential candidates to be used as model species in toxicology. It is well accepted that the copper transporter (Ctr-1) and Cu-ATPase (ATP7) membrane proteins primarily regulate copper uptake and transport in vertebrates. Using Polymerase Chain Reaction (PCR) with degenerated primers, cloning and sequencing approach we sought for *P. vivipara* nucleotide sequences homologous to those key genes involved in copper homeostasis. Ctr-1 is an integral membrane protein composed of three domains and involved in copper uptake. We identified a 139 nucleotides sequence for Ctr-1 in *P. vivipara*. This sequence showed 92% identity when compared to the known corresponding region of the Ctr-1 of the cyprinodontiform *Fundulus heteroclitus*. This allowed us to do the preliminary annotation of this new sequence. Also, a 266 nucleotides sequence was found for ATP7 in *P. vivipara*. This sequence shared 74 and 81% identity with the corresponding region of the ATP7A and ATP7B of the perciform *Sparus aurata*, respectively. Altered levels of environmental copper are assumed to be associated with changes in the transcript levels for the identified genes. RACE methodology is now being used to obtain the full-length sequences for Ctr-1 and ATP7 in *P. vivipara*. Also, copper exposure experiments to characterize the regulation of the new identified genes at the transcriptional level are being performed using RT-qPCR. [Supported by Brazilian CNPq (INCT-TA) and CAPES (Ciências do Mar)].

**54 Effects and Adaptations in Estrogen Signaling Pathways in New Bedford Harbor Killifish** G.V. Callard, S.R. Greytak, K.A. Cotter, Boston Univ, Biology; D. Nacci, USEPA, ORD, NHEERL, Atlantic Ecology Division. Reproducing the complexities of environmental contamination in a laboratory setting has been a critical barrier to understanding long term effects and adaptations to environmental endocrine disrupting chemicals. The killifish population at a highly contaminated Superfund site (New Bedford Harbor, MA; NBH), in comparison with killifish at a nearby clean



site (Scorton Creek, MA; SC), has proven to be an extremely valuable resource. The history of pollution in NBH indicates exposure to PCBs, heavy metals and other contaminants for ~50 yr (~20 generations). Survival has been ascribed to acquired resistance to dioxin-like chemicals that induce toxicity through AhR signaling pathways but survival alone cannot account for the reproductive success of the NBH population. Molecular markers and standard reproductive parameters indicate that NBH fish are exposed to an estrogenic environment and display evidence of endocrine disruption. To investigate the possibility that chronic exposure to NBH pollutants leads to adaptations in estrogen signaling pathways, we cloned and characterized the two *Cyp19* (aromatase) and three *Esr* (estrogen receptor) genes in killifish. Despite a high degree of polymorphism in sequences of all five genes, no obvious site-related changes were identified. Paradoxically, NBH as compared to SC larvae overexpress *ERα* mRNA 5-fold; however, they are hyporesponsive to administered estrogen, as measured by inducibility of the *ERα* gene. One interpretation for this discrepancy is that the *ERα* mRNA pool in NBH fish has a large fraction of mRNAs that are untranslatable or encode non-functional isoforms. Although our present understanding of endocrine disruption focuses on dysregulation of transcription, advances in molecular endocrinology indicate that transcription and splicing are functionally coordinated and regulated. Currently, we are testing the hypothesis that exposure to environmental chemicals can impact splicing decisions on estrogen responsive genes, and thereby alter the quantity and quality of *ERα* transcripts. Whether dysregulation of *Esr1* splicing is adaptive in an estrogenic environment and somehow related to the hyporesponsiveness phenotype of NBH fish remains to be investigated. Supported by research grants from NOAA (WHOI) National Sea Grant College Program and NIEHS P42ES07381.

**55 Coupling Diffusive Gradient in a Thin Film Probe and IC-ICP-MS for the Simultaneous Determination of  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$**  Y. Hong, Johns Hopkins Univ, Geography and Environmental Engineering; E. Rifkin, Nonaqua Aquarium Baltimore, National Aquarium Conservation Center; E. Bouwer, Johns Hopkins Univ, Geography and Environmental Engineering. Mercury (Hg) is a metal contaminant that is highly toxic, globally ubiquitous, and environmentally persistent. A great need exists for monitoring  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  concentrations in environments for assessing the risks associated from Hg in ecosystems. However, analysis of Hg is challenging due to lengthy analytical procedures, numerous potential artifacts, and the high cost of the usual techniques that employ USEPA Methods 1630 and 1631 for  $\text{CH}_3\text{Hg}^+$  and total Hg, respectively. As an alternative to the current Hg monitoring procedures, we combined a diffusive gradient in thin film technique (DGT) and ion chromatography – inductively coupled plasma – mass spectrometry (IC-ICP-MS) for the in situ measurement of labile  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  in water. Both  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  diffused through an agarose diffusive layer and accumulated in a resin layer, then the mercury species were extracted using acidic thiourea solution to form stable thiourea-Hg complexes that were separated and detected via a cation exchange column and ICP-MS. The effective diffusion coefficients for the  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  complexes in the agarose diffusion layer in the presence of chloride and DOC were evaluated from laboratory experiments. The DGT probes were deployed in several different water bodies, such as river, estuarine, and saline environments. The method detection limits are 0.1 and 0.5 ng L<sup>-1</sup> for  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$ , respectively, for 4 weeks deployment with 0.5 mm diffusive layer. The DGT coupled to IC-ICP-MS technique can measure time averaged labile  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  concentrations simultaneously in water and can be used to study the bioavailability of the mercury species, making it useful as an in situ monitoring tool.

**56 PAH Analytical Techniques and Impacts on Risk Assessment** E.L. Martin, CH2M HILL Canada; C.D. Sandau, TRIUM Environmental Inc. Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of chemicals that are formed during incomplete combustion of organic substances. They can be anthropogenic in origin – burning of coal, oil & gas, tobacco – or form naturally in the environment – volcanoes, forest fires. Due to the numerous sources, PAHs are ubiquitous in the environment. PAHs are often found at contaminated sites. They are a contaminant of concern at approximately 60% of National Priorities List (NPL) sites and are ranked eighth on the 2007 CERCLA priority list of hazardous substances. A number of standard laboratory techniques are available to identify the composition, distribution, and abundance of PAHs in various environmental media including the United States Environmental Protection Agency

(USEPA) method 8270C which uses gas chromatography/mass spectrometry (GC/MS). However, more sensitive techniques for measurement of PAHs have also been developed including isotope dilution high-resolution mass spectrometry (ID-HRMS). We have conducted a study to compare a standard analytical method (EPA Method 8270C) to a more sensitive method (ID-HRMS) for monitoring PAHs in soil. Our results indicated that the frequently used standard laboratory methodology (EPA 8270C) consistently overestimated PAH concentrations in soil as compared to the more sensitive ID-HRMS technique which was found to be more accurate and more precise. Using the benzo[a]pyrene toxic equivalency (BaP TEQ) scheme, it was found that the BaP TEQ values generated using the standard laboratory technique were much greater than those from the more sensitive technique. These findings may have important implications for contaminated site risk assessment. Risks associated with exposures to PAHs in soil (via inadvertent ingestion, dermal contact and inhalation of soil/dust) are directly proportional to measured PAH concentrations. Any errors/inaccuracies in the determination of PAH concentrations will consequently impact the calculation of risk. Thus, use of more sensitive laboratory techniques for PAH measurements will reduce uncertainty and provide for a more accurate understanding of site conditions and liability.

**57 A Sensor Array for Rapid Screening of Dissolved Hydrocarbons in Sea Surface Waters** A. Ross, X. Qi, E. Crooke, C. Stalvies, D. Fuentes, S. Armand, A. Revill, A. Taluker, CSIRO. Recent oil release events at Deepwater Horizon in Gulf of Mexico block MC252, and Montara in Australia, have outlined the need to rapidly survey the hydrocarbon content of near surface water over large geographic areas of the ocean surface in order to measure water quality. Conventional oceanographic approaches involve water sampling and measurement at predetermined stations, often through the deployment of conductivity, depth and temperature (CTD) profilers with attached Niskin bottle water sampling apparatus. The analysis of water samples for hydrocarbons can use a number of extraction methods followed by gas chromatography or gas chromatography and mass spectrometry. These methods and analysis permit the detailed determination and quantification of individual gaseous, volatile and higher molecular weight hydrocarbons compounds, however analysis can take several hours. Hydrocarbon sensors have been recently developed for marine applications. These sensors have both low hydrocarbon detection limits in the parts per million to parts per trillion range and rapid measurement cycle times in the order of seconds to minutes. In addition the devices are ruggedized for field deployments. These sensors include fluorimeters tuned for the detection of poly-aromatic hydrocarbons (PAH) and sensors for volatile organic compounds (VOC) tuned for the low molecular weight hydrocarbons. These sensors offer the potential to provide rapid, spatial and temporal sampling and detection; however they do not provide detailed hydrocarbon compositional data. Separate sampling and analytic techniques for hydrocarbon analysis are described in the accompanying poster. In this paper the design and implementation of an arrayed sensor system in an underway configuration combined with onboard complimentary conventional analysis is described. In response to the Deepwater Horizon oil release a hydrocarbon sensor array system comprising Chelsea Aquatracker, Contros and Trios fluorimeters was deployed and operated for over 80 days in the Gulf of Mexico. Data was acquired covering over 8,998 linear nautical miles (~16,664 line kms) from June 5<sup>th</sup> to the September 15<sup>th</sup> 2010 during and after the MC252 spill. This data was mapped and reported to the Unified Area Command daily. During this time the system was successfully operated in various sea states and in environments ranging from riverine to oceanic waters, and from pristine waters through to waters with oil slicks at the surface.

**58 Laboratory Processing of Incremental Samples: Options and Data Effects** M. Bruce, TestAmerica, Technical Director; M. Heskett, L. Penfold, TestAmerica. The Interstate Technology and Regulatory Council (ITRC) team on Incremental Sampling Methodology (ISM) has been working for nearly three years to assemble guidance to improve the quality of site characterization. The ISM guidance document public release and training sessions are planned for early 2012. This presentation provides an overview of the current status on the near final ISM guidance related to laboratory sample processing options and how analytical data is affected by the various processes. ITRC is a state run organization that develops consensus guidance for environmental issues. Alaska and Hawaii have widely adopted the use of ISM for site characterization. Other states such as Missouri and Ohio have used ISM on limited number of sites. A few states [Texas, Florida, New

Jersey, Michigan, Wisconsin and Iowa] currently have regulations that prohibit the use of composite based sampling techniques such as ISM. The Dept of Defense and US Army Corps of Engineers in particular has devoted many resources to the development of ISM and is also a significant contributor to this ITRC team. In addition to other state and federal regulatory representatives, this guidance is also being developed by engineering firms, consultants and state, Univ, government and commercial laboratories. The overall goal of ISM is to provide an accurate estimate of the mean concentration for each contaminant of concern within each decision unit. SW-845 Method 8330B is the best known guidance for laboratory processing of incremental samples. The ITRC guidance includes other analyte groups such as metals, PCBs, volatile organic compounds and semivolatile organic compounds. The specific laboratory processes used affect the retention of analytes, contamination from sample processing equipment and other samples and subsampling representativeness. These processes have different effects on the final analytical data. This presentation will summarize the low bias, high bias and precision effects of the moisture modification, grinding and subsampling processes. The laboratory processing options include air drying, water addition, field moist options, freeze drying, disaggregation, milling, fractional scoops, riffle splitting, rotary sectorial splitter, 1 and 2-dimensional slabcake. Application of standard quality assurance principles is discussed as well laboratory certification options.

#### 59 Partitioning of PCBs in Neutral Lipids, Polar Lipids and Proteins of a Fish

**K. Maenpää**, M. Leppanen, Univ of Eastern Finland, Dept of Biology; F. Tigistu-Sahle, Univ of Helsinki, Dept of Biosciences; K. Figueiredo, Univ of Eastern Finland, Dept of Biology; R. Kakela, Univ of Helsinki, Dept of Biosciences. Lipids are the dominant force in determining organic contaminant accumulation in aquatic organisms because hydrophobic organic chemicals (HOCs) partition mainly into lipid phase. Therefore, lipid normalized tissue concentrations have helped to reduce variation in tissue residues between different species and aid in understanding of contaminant toxicity and distribution in food webs. However, partitioning of contaminants between different lipid classes is not very well known. Lipid composition contributes to variation in bioaccumulation potential and neutral storage lipids are thought to be the most important lipid class for HOCs. On the other hand, polar membrane lipids are the target for HOC toxicity and knowing the concentration in membrane lipids would greatly improve the risk assessment of HOCs. We have applied silicone (polydimethylsiloxane) equilibrium approach to estimate silicone in olive oil, phosphatidylcholine and albumin partition ratios for carbon 14 labelled PCB-153 and PCB-77 in purpose to characterize HOC distribution in storage lipid, membrane lipid and protein fractions. PCB distribution was dominated by olive oil (78–82%) while phosphatidylcholine (11–20%) and albumin (2–8%) had lower share. The method is tested with the extracted native PCB contaminated pikeperch oil. Simultaneously, we will determine storage lipid, membrane lipid and protein fractions in fish. If the native and labelled PCBs show the same equilibrium ratios in silicone fish oil system, we can calculate PCB concentrations for all native congeners in these three main sorbing phases. The approach can be applied in dose-response studies of membrane toxicity and bioaccumulation models.

#### 60 Low-cost High-throughput Analytical Method for Polychlorodibenzo-*p*-dioxins, Polychlorodibenzofurans and Polychlorobiphenyls in Fish Tissue

**B. Subedi**, Baylor Univ, Dept of Chemistry and Biochemistry, Dept of Biology, Dept of C; **S. Usenko**, Baylor Univ, Dept of Environmental Science. To increase the samples throughput, enhanced pressurized liquid solid phase extraction was developed incorporating pressurized liquid extraction and all independent clean-up techniques. USEPA methods of polychlorodibenzo-*p*-dioxins, polychlorodibenzofurans (PCDD/Fs), and polychlorobiphenyls (PCBs) analysis in fish tissue include the independent silica gel, fluorsil, alumina, and celite/carbopak column cleanup techniques following extraction. Under improved method, fish composites (10 g) were extracted and cleaned simultaneously using alumina, fluorsil, silica gel, celite, and carbopak (10:10:5:5:0.5 g/g). Cleaned extracts were concentrated and analyzed through high resolution gas chromatography negative chemical ionization mass spectrometry. Adsorbents and solvents were optimized to separate PCDD/Fs and PCBs. The average recoveries ( $n=3$ ) of individual PCBs in dichloromethane: hexane (1:1 v/v) extracts were within 90–103  $\pm$  4.7% and individual PCDD/Fs in toluene extracts were within 72–100  $\pm$  7.3%. Samples preparations time and solvents were reduced as much as 80% and 67%, respectively.

#### 61 Buoy-Borne, Real-Time Sensing and Time-Resolved Sampling Of Hydrophobic Organic Chemicals In Onondaga Lake

**R. Oakes**, State Univ of New York, College of Environmental Science and Forestry, Dept of Chemistry; J.P. Hassett, SUNY College of Environmental Science and Forestry, Dept of Chemistry; J.M. Croskrey, SUNY College of Environmental Science and Forestry, Dept of Chemistry; M.M. Giardono, D. Salley, SUNY College of Environmental Science and Forestry, Dept of Chemistry. Traditional methods for extracting and detecting trace organic contaminants in water are limited in many ways. Grab samples only represent one moment in time, limiting their temporal scope, and a large amount of time and effort is needed to collect enough water to achieve the needed low detection limits. This research utilizes a submerged, stirred extraction cell containing small diameter, therefore high surface area to volume ratio, semi-permeable membrane polydimethylsiloxane (PDMS) tubing to extract and concentrate dissolved hydrophobic contaminants in situ. Hydrophobic compounds dissolved in the water diffuse across the membrane into a solvent stream. Diffusion rate is equivalent to 17 L/day of water extracted, achieving a concentration factor of nearly 200. The extract is pumped through an on-board absorbance and fluorescence detector for real-time detection and into 24 hour composite collection vials for retrieval and laboratory analysis. Two solar-powered buoy-borne systems that generated real-time data on the presence and concentrations of trace hydrophobic organic contaminants were deployed in Onondaga Lake, Syracuse, NY, from August to December of 2010. One buoy was located at the southern end of the lake downwind of a chlorobenzene source and above sediments contaminated with PAHs, gasoline components, and coal tar. Data were collected continuously onboard the buoys and transmitted via radio to shore. Absorbance and fluorescence data demonstrated that during high wind events, the wave action disturbed the sediments and increased the dissolved concentrations of these contaminants in Onondaga Lake. This was further confirmed by GC-MS analysis of the composite extracts, which also provided information on individual compounds.

#### 62 Evaluation and Calibration of a Rapidly Equilibrating Polymer Coated Glass (POG) Sampler for Screening Priority Chemicals

**S. Genu-aldi**, Environment Canada, Science and Technology Branch; T. Harner, Environment Canada, Atmospheric Science and Technology Directorate. Polymer coated glass samplers (POGs) consist of a thin layer of ethylene vinyl acetate (EVA) coated on a glass fiber filter or aluminum foil substrate. Calibration of the sampler was performed through an eight week indoor uptake study using six different film thicknesses ranging from < 0.1 to 20  $\mu$ m. Actual air concentrations were measured using weekly low-volume air samples, polyurethane foam (PUF) disks, and sorbent-impregnated polyurethane foam disks (SIPs). Depuration compounds were coated onto the POG sampler prior to deployment and used to calculate sampling rates. These consisted of labeled polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and perfluorinated compounds (PFCs). The samplers were also evaluated for their use as a rapid screening tool for assessing exposure to PCBs, OCPs, PFCs, and siloxanes in indoor air. Analysis was performed using thermal desorption gas chromatography/mass spectrometry (TDS-GC-MS). The POG shows promise due to its relatively low cost and ease of deployment and its applicability to a range of priority chemicals. The ability to alter the film thickness and hence the sorption capacity of the POG allows for a versatile sampler that can be used under varying sampling conditions and deployment times.

#### 63 Observation-based Distribution, Inventories and Fluxes of PCBs in and Across Key Arctic Ocean Compartments

**O. Gustafsson**, A. Sobek, D. Carrizo, Stockholm Univ. The predictability of future distribution and exposure of PCBs in the Arctic hinges centrally on the quality of our understanding of the present-day distribution and processing of PCBs in the Arctic system. The first part will present pan-Arctic observations of the PCB distribution and inventories in key compartments such as the polar mixed layer, sea ice, shelf sediments and all the major subsurface water masses. Next, observation-derived fluxes across key system boundaries such as surface ocean settling fluxes, pan-Arctic river export and shelf sediment burial will be presented. A synthesis of these results will provide information on their internal geochemical/budget consistency. Finally, the plausible effect of Arctic change scenarios on PCB distribution and fate will be discussed in the context of our present understanding of PCBs in the contemporary Arctic Ocean system.

**64 A Global 3-D Model to Simulate Long-range Transport of PAHs: Application to Arctic Contamination** C. Friedman, Massachusetts Institute of Technology, Center for Global Change Science; N.E. Selin, Massachusetts Institute of Technology, Engineering Systems Division. We simulate the long-range transport of polycyclic aromatic hydrocarbons (PAHs) to the Arctic under present and future climate using a global 3-D chemical transport model (GEOS-Chem). PAHs reach the Arctic environment by long-range atmospheric transport. We present initial results from model development for the PAHs phenanthrene, pyrene, and benzo[a]pyrene, focusing on episodic transport to the Arctic and sensitivity to temperature and other meteorological parameters. This work represents the first effort at modeling PAHs globally taking into account episodic transport events. Results are compared with previous efforts to model atmospheric transport of PAHs, which have either relied on multimedia box models or regional models focusing on specific sources or receptors, or used coarse-scale general circulation models (GCMs) not capable of capturing individual meteorological events that could lead to episodic transport from specific sources. Improved parameterizations of gas-particle partitioning, atmospheric reactivity, and wet and dry deposition are developed. Initial results show that model-predicted annual average concentrations correlate well with land-based measured concentrations for phenanthrene, pyrene, and benzo[a]pyrene, but are biased low. We apply these results to identify the importance of various remote source regions to particular Arctic locations compared to local sources, and to calculate the importance of episodic transport to the Arctic. We additionally show results for temperature sensitivity and initial calculations for PAH transport to the Arctic in a changing climate.

**65 The Role of Snow and Ice on the Fate of Persistent Organic Pollutants in the Arctic Marine Environment** C. Halsall, Lancaster Univ, Lancaster Environment Centre; S. Del Vento, G.P. Codling, Lancaster Univ, Lancaster Environmental Centre; L. Ahrens, Environment Canada, Science and Technology Branch; R. Ebinghaus, GKSS Research Centre. This presentation will explore current knowledge on the behaviour of chemical pollutants in sea-ice and sea-ice snow, placing concentrations in context with other studies and examining the role of ice in delivering pollutants to marine waters. During the International Polar Year there were several field-based programmes that examined persistent chemicals in snow and ice in the Arctic. These studies were undertaken for different purposes, either (1), to construct pollutant time-series to relate accumulation in ice with known chemical use/emissions; or (2), to examine chemical transfer processes in transient seasonal snow and sea-ice. Understanding (2) is particularly important in the context of a 'warming Arctic' as changes to snow and ice systems will in turn affect the timing and loading of pollutants to surface waters. Results from field work conducted in the Canadian Arctic show the presence of PBDEs, PCBs, OC pesticides and C5-C13 perfluorocarboxylates (PFCAs) and PFOS in the remote marine snowpack at levels comparable to other recent studies. Levels of hydrophobic contaminants in wind-blown snow measured from late winter to early melt are, in the main, related to the physical properties of the sampled snow, rather than seasonal changes in air temperature ( $-25^{\circ}\text{C}$  to  $0^{\circ}\text{C}$ ) with little evidence to support a hypothesis that contaminant levels change markedly in sea-ice snow with increasing air temperatures prior to melt. Concentrations of hydrophobic POPs measured in snow and sea-ice (expressed as melt-water equivalents) show marked enrichment compared to levels measured in beneath-ice seawater (ratio of snow-ice/seawater) although this enrichment declines as melting commences. In contrast, several PFCAs, including PFOA, were found at surprisingly high concentrations in beneath-ice seawater both prior to and during ice-floe breakup, with levels of PFOA up to  $300\text{ pg/L}$  in some samples, comparable to or higher than concentrations found in the overlying snowpack and  $\sim 3$ -fold higher than observations in mid-Atlantic surface water. A small number of first-year sea-ice samples also revealed high concentrations of PFCAs, implying a possible enrichment process at the ice-seawater interface. The results of these findings will be extrapolated in the context of ongoing changes to the cryosphere, with the loading of chemicals attributable to marine snow and ice compared to direct deposition to 'ice-free' waters. Areas of uncertainty will be highlighted.

**66 Accumulated Contaminants in Ice Cores from Svalbard: Considerations of a Future Source to the Environment** M. Hermanson, Univ Center on Svalbard, Arctic Technology; E. Isaksson, Norwegian Polar Institute; C. Teixeira, Environment Canada; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, National Water Research

Institute. Since 1998 we have analyzed various persistent organic contaminants in ice cores collected from two ice fields on Svalbard, including Høltedahlfonna (2005) to the west and Austfonna (1998) to the east, about 220 km apart, roughly west-east. The results from Høltedahlfonna, the most comprehensive data set, include analysis of 220 different compounds in four compound classes including PCBs, BFRs, current-use (CUP) and legacy pesticides (LP), and halobenzenes. Compounds from these groups cover vapor pressures (VP) ranging over 8 orders of magnitude (from  $2.87 \times 10^{-1}\text{ kPa}$  to  $2.95 \times 10^{-9}\text{ kPa}$ ), and include compounds considered to be too volatile to be deposited (1,3-dichlorobenzene) and others once thought to be too non-volatile for long-range transport (decabromodiphenyl ether, BDE-209). Compounds observed at Høltedahlfonna also have OH. reaction rates suggesting atmospheric lifetimes ranging from one day (Chlorpyrifos) to nearly one year (g-HCH), the former usually considered to be too short for long-range transport. All of these 4 compounds, representing extremes of VPs and atmospheric lifetimes, are present in greater abundance than nearly any other throughout the Høltedahlfonna core (and in greater abundance than  $\Sigma\text{PCB}$ ), showing that a wide range of organic contaminants needs to be considered when postulates are made of what contaminants will be released if this ice melts, and in what environmental matrix they will be found. Results from Austfonna, which includes only pesticides, suggest variability in net contaminant deposition: in comparison to Høltedahlfonna, where 9 of 47 analyzed CUPs were found, Austfonna shows net deposition of 21 CUPs of 26 analyzed. The most abundant of the CUPs found at Austfonna and not Høltedahlfonna includes a series of neurotoxic organophosphorus (OP) pesticides. Our investigations into sources using long-term (10-year) average air mass trajectories show seasonal and long-term shifts in source regions to these two sampling sites, suggesting a high level of complexity in evaluating the burden of organic contaminants on Svalbard that will be released as these ice masses melt.

**67 Bioaccumulation of POPs in a Top Predator, the Osprey, in Alpine Regions of Western Canada** J.E. Elliott, Environment Canada, Pacific Wildlife Research Centre, Science and Technology Branch, Canadian Wildlife Service; M. Guigueno, K.H. Elliott, J. Levac, Univ of Manitoba; M. Wayland, Environment Canada; C.A. Morrissey, Univ of Saskatchewan; D.C. Muir, Environment Canada. Due to climate change, chemicals that accumulated in glaciers through evaporation from warm regions and subsequent condensation in cold regions are being released into alpine environments. To examine the role of glacial geography in determining contamination of wildlife in alpine ecosystems, we measured persistent organic pollutants and mercury in apex predators, ospreys (*Pandion haliaetus*) breeding in 16 watersheds in western Canada. Despite living in environments far from point sources of contamination, ospreys in Canadian alpine lakes had significant levels of DDT, PCBs, toxaphene and mercury. After accounting for proximate factors such as trophic level and lipid content, HCB, chlordane, mercury, PCBs decreased with watershed size and toxaphene, DDT, HCB and mercury decreased with watershed size divided by lake size. Once trophic level was factored, ospreys feeding in large watersheds that drain into relatively large, lower elevation lakes with less glacial input had higher levels of contamination. Toxaphene, mercury, HCB, and DDT all decreased with the amount or proportion of glaciation while toxaphene and chlordane decreased with elevation. Thus, foraging ecology (trophic level) and geography explained significant portions of the large variation in osprey contaminant levels in western Canada, but the geographical variation was not consistent with the idea that the patterns in contaminants were determined by current long-range transport or glacial melt. Rather, we suggest that contaminants largely melt out during annual snow pack melt and bioaccumulate at lower elevation lakes, especially lakes with poor drainage. Our study highlights the importance of understanding how biological processes integrate physical patterns when studying the environmental chemistry of wildlife.

**68 Empirical Investigation into the Fate of Organic Contaminants During Snowmelt in a Sub-Arctic Snowpack** C. Halsall, Lancaster Univ, Lancaster Environment Centre; G.P. Codling, Lancaster Univ, Lancaster Environmental Centre; M. Bergknut, Umea Univ; K. Wiberg, Univ of Umea, Dept of Chemistry; S. Del Vento, Lancaster Univ, Lancaster Environmental Centre; H. Laudon, Swedish Univ of Agricultural Sciences (SLU), Dept of Forest Ecology and Management; M. Tysklind, Univ of Umea, Dept of Chemistry. To understand the role of the seasonal snowpack in providing atmospherically derived organic contaminants to northern catchments a study was undertaken at the Krycklan Catchment Study



(KCS) site in northern Sweden (64°14'N, 19°46'E). For the KCS, snowmelt provides ~66% of the annual stream flow and is therefore likely to make a significant input to contaminant loads to catchment headwaters. The fate of snow-associated contaminants during the melt period is not fully understood, with some chemicals possibly undergoing a first 'flush' during initial melt while others may re-partition back to the air or to particulate matter and hence be retained in the pack until final melt. In this study two approaches were taken: (i) a doping study utilising a group of chemicals of varying physical-chemical properties (PCBs, OC pesticides, PBDEs, organophosphorus insecticides and perfluorosulphonates (PFSAs)) that were artificially introduced into a surface snow layer of an aged snowpack and their movement tracked every 24 hours over a five day period; (ii) a time-series of 'native' contaminant concentrations in snow (PCBs, OC pesticides, PBDEs and PFCAs) achieved over 14 days during the melt period. From the doping study (conducted in semi-enclosed metal tubes sunk vertically into the snow) mass recoveries of the chemicals from the snow and captured meltwater were in the order of PFSAs>PBDEs>PCBs=OCs, indicating that volatile compounds can be lost to the atmosphere. Chemical movement down the snow column was rapid for several of the 24 h periods, with an even distribution of chemicals down the length of the snow column as well as detected in meltwater collected at the base of the snowpack. For the 'native chemicals', PCBs showed a decline from >1 ng/L (in fresh snowfall) to <0.3 over the 14 day period with most of the decline attributable to the loss of tri- and tetra-chlorinated homologues. Levels of PFOA ranged from <10–200 pg/L and PFOS <3–100 pg/L, with concentrations generally higher at the base of the snowpack. Unlike PCBs, concentrations of PFOS, PFOA (and other PFCAs) did not decrease uniformly in the melting snowpack and were retained right up to final melt with implications regarding the extent and timing of chemical release to meltwater runoff.

#### 69 Partitioning of Persistent Organic Pollutants Between Sediments and Benthic Deposit Feeders in Western Antarctic Peninsula L. Zhang,

Univeristy of Rhode Island, Graduate School of Oceanography; R. Lohmann, Univ of Rhode Island, Graduate School of Oceanography. Studies have shown that previously deposited persistent organic pollutants (POPs) are being released from glaciers due to global warming. The melting glaciers are believed to be the secondary source of POPs to the Western Antarctic Peninsula (WAP) coastal regions. These POPs have been found to bioaccumulate along the food web into Antarctic penguins which body levels of DDTs have not declined since 1970s. Hydrophobic POPs should not only be present in the pelagic food web, but also be transported downward with sinking particulate organic matter into sediments. Thus, it is important to investigate the concentrations of POPs in the sediments of the WAP and their transfer along the benthic food web. Benthic biota can take up POPs through contact with porewater and/or via ingestion of sediment particles. The uptake pathway is closely related to the animals' feeding strategies. Previous studies have found at certain locations that the partitioning of POPs between filter feeding organisms and porewater were at equilibrium and thus POPs porewater concentration can be used to predict body burdens at that specific site. This might not be true for deposit feeders which not only continuously exchange POPs with surrounding porewater, but also ingest sediment particles indicating a potential for bioaccumulation. As part of an ongoing collaborative research to study the impact of climate change on the transport of POPs in the Antarctic marine food web, surface sediments (0–5 cm depth) and subsurface/ surface deposit feeding holothurians samples were collected at five different locations from north to south along WAP. PCB concentrations (12 congeners) were highest at the tip of WAP at 0.5 ng/g dry weight and decreased towards the interior of Antarctic continent to 0.03 ng/g. However, the PCB concentrations in the lipids of deposit feeders did not reflect this trend. Porewater concentrations were assessed with passive samplers to determine if an equilibrium partitioning model can be used to predict POP concentrations in benthic deposit feeders in WAP. The results provide a better understanding of the transfer of POPs into the Antarctic benthic food web as well as the site-specific risk assessment of sediments.

**70 Indoor Dust and the Pet Dog: PBDEs, PCBs, and Pesticides** C. Orazio, US Geological Survey, Columbia Environmental Research Center; C. Grimm, Smithsonian National Zoological Park, Center for Species Survival; N. Diggs, Smithsonian Migratory Bird Center, National Zoological Park, Smithsonian Conservation Biology Institute; S. Tan, Smithsonian National Zoological Park, Center for Species Survival; K. Feltz, K. Echols,

US Geological Survey, Columbia Environmental Research Center; P. Marra, Smithsonian Migratory Bird Center, National Zoological Park, Smithsonian Conservation Biology Institute; P. Peterman, US Geological Survey, Columbia Environmental Research Center. House dust and blood serum from the family's pet dog were collected from rural and suburban homes near Washington DC to assess levels of polybrominated diphenyl ethers (PBDEs), PCBs, and organochlorine pesticides. Pre-weighed clean dry glass fiber filters were used to swipe dust from high surfaces. Care was taken to prevent dust contamination of the dog blood samples ( $n=18$ ) during collection and analysis. The breed of dog, sex, age (2–13 years), weight (13–90 lbs), dog food (not analyzed in this study), current medications, daily time spent outdoors (0.25–12 hrs), and dog bedding material were recorded. Dust (0.013–0.055 g) and blood serum (3 g) samples were processed separately, accompanied by appropriate quality control samples, including blank spiked filters and bovine serum blanks. Following extraction, purification and fractionation, the dust and blood extracts were analyzed by GC/MS and isotope dilution GC/high resolution MS for 41 PBDE congeners, 21 pesticides, and PCB congeners. As expected, based on other dust studies, the levels and patterns of PBDE congeners in the house dusts varied. PBDEs were at quantifiable levels in 12 of the 18 dog blood samples, with PBDE-47 and PBDE-209 levels that ranged from 16–80 and 60–300 ng/g lipid-wt, respectively. The predominant PBDEs in the dog bloods were 47, 99, 100, and 209. A linear trend existed for dog blood and dust levels of PBDE 209. The highest level of PBDE-209 in dust of 11,100 ng/g corresponded to 300 ng/g lipid-wt in the dog blood. Dust levels of total-PCBs ranged from 20–3168 ng/g but were less than 2,000 ng/g lipid-wt in the dog blood samples. Three of the 18 dogs had detectable residues of chlordane: The dog from the home with the highest indoor dust level of total-chlordane (22,000 ng/g) had the highest blood level of oxychlordane (219 ng/g lipid-wt). Exposure to PBDEs, PCBs, and pesticides via the diet also can contribute to residue levels in the dog blood; recently Venier and Hites (2011) reported that different brands of dog food do not play an important role in the concentrations of PBDEs. The results of our study indicate that levels of certain contaminants in indoor dust correlate to levels in the family dog.

#### 71 Evaluating Exposure to BDE-47 in Homes: Impact of Couch Construction, Temporal Variability and Variability in Measurement

**Methods** D. Bennett, UC Davis, Public Health Sciences. BDE-47 is used as a flame retardant in furniture foam. Our objectives were to determine 1) if the type of furniture upholstery in a home influences the air concentration of BDE-47 measured in an air sample collected in a home, 2) determine the temporal variability in air and dust concentrations, and 3) determine the differences in measured concentration in dust by measurement method. We quantitatively measured bromine in furniture and determined BDE-47 air and dust concentrations at multiple time points in California homes. First, a portable XRF (X-ray fluorescence analyzer) was used in 68 homes, measuring bromine to a depth of approximately 0.5 cm, representing the foam concentration when placed on the surface. Multiple sub-sections (i.e., seat and back cushions, backing, throw pillows) of both the largest and most frequently used (if different) couch or chair in the main living area were tested, with a preliminary mean bromine concentration of 7030 ppm (SD = 8220). Bromine concentrations, multiplied by the respective section's surface area, were summed to calculate the potential source strength. As an alternative, a small sample of foam from the seat cushion was removed if accessible (i.e., there was a zipper) and the participant agreed (52% of households) and analyzed by a semi-quantitative Elisa method to determine if BDE-47 was present (72% of samples above LOD). A passive air sampler (PUF) was left in the home for 30 days and the PUF was extracted and analyzed for BDE-47 (preliminary mean concentration of 61.4 mg/m<sup>3</sup> (SD=84)). To analyze the data, homes were divided into two groups: (1) those with couches with a thicker resistance layer between the cushion and air, either leather (24% of homes) or an additional upholstery liner (18% of homes), and (2) all others. The slope of the correlation between the bromine source strength and the BDE-47 air concentration was lower for those homes categorized as having a thicker resistance layer, suggesting the potential for less exposure. Further studies should be conducted to determine if XRF combined with couch construction may be a useful, low-cost indicator of exposure and applicable to other flame retardants. Second, air and dust measures were collected approximately 1 year apart in 56 homes to evaluate temporal variability. Third, measured concentrations will be compared between two different investigator collected methods and between an investigator collected method and vacuum cleaner samples.



**72 Characterization of Two Chiral Current-use Residential Pesticides in the Indoor Environment** E.M. Ulrich, US Environmental Protection Agency, National Exposure Research Laboratory, Methods Development and Applications Branch; Q. Wang, Student Contractor to the USEPA; D. Stout, USEPA. Pesticides are applied to the interior of homes, schools, places of business and employment to control a variety of pests. Residential pest control practices may result in chemical residues located in close proximity to daily activities and potentially serve as a source of exposure. The primary routes of exposure after indoor pesticide application are inhalation, dermal, and ingestion all of which contribute to the aggregate exposure of occupants. Crack and crevice application of several current-use pesticides has allowed evaluation of important exposure routes, fate and transport within the unoccupied USEPA Indoor Air Research House in North Carolina. Two of these pesticides, fipronil and cis-permethrin, were studied to determine temporal and spatial trends of their enantiomers. Many pesticides, including fipronil and permethrin, are typically formulated and applied as a racemic mixture (equal amount of enantiomers). Differences in the pesticidal efficacy of enantiomers may offer an opportunity for "greener" enantiomer enriched formulations which allow for an equally effective but lower concentration of active ingredient. Because enantiomers can have differential toxicity, it is important to thoroughly understand exposure to accurately assess the potential risk from different enantiomers. Chiral pesticides are not frequently studied in indoor settings, creating a data gap for exposure and risk assessments. In this study, selected carpet, surface wipe, indoor air, and deposition coupon samples collected in several rooms of the Research House were analyzed using enantioselective gas-chromatography/mass spectrometry. Preliminary analysis of the enantiomer fraction (EF) data reveals that both pesticides were primarily racemic or near racemic (fipronil EF= 0.47–0.51; cis-permethrin EF= 0.45–0.55). Indoor environments are not thought to have many opportunities for enantioselective degradation or processes, which seems to be confirmed by these findings. However, cis-permethrin had a broader range of EF values, which may indicate some enantioselective degradation, which is usually attributed to biological interactions. Temporal and spatial trends of the EF values will also be discussed.

**73 Traditional and Forensic Signatures of Indoor Air** S. Emsbo-Martingly, New Fields Environmental, Forensics Practices, LLC; A. Uhler, NewFields. Environmental scientists recognize the highly variable nature of indoor air. It is greatly affected by the life style of the human inhabitants, the building materials, the quality of the outdoor air, and, occasionally, vapor intrusion. This presentation features a series of case studies that use patterns of TO15 analytes to contrast human activities in residential, recreational, commercial, and industrial environments. The differentiation of source signatures benefits greatly from TO15 methods (GC/MS) designed for traditional and forensic hydrocarbon analytes with trace level detection limits. Of particular interest is the close between outdoor and indoor air. In addition, the case studies help demonstrate how the effects of environmental weathering help identify the dominant sources of indoor air contaminants. The compounds that appear in outdoor and indoor air include benzene, toluene, ethylbenzene, xylenes, styrene, naphthalene, indane, indene, and many other aliphatic, aromatic, and heteroatomic volatile organic compounds (VOCs). These data emphasize the importance of outdoor air sampling and help optimize the sample collection and data interpretation strategies.

**74 Relationship Between House Dust and Indoor Air Concentrations: Applying Partitioning Theory to Empirical Data** R. Dodson, L. Perovich, J. Brody, R. Rudel, Silent Spring Institute. Individuals encounter a wide range of pollutants in indoor environments, and semivolatile organic compounds (SVOCs), which are found both in the gas and condensed phase, are of particular interest because they can be redistributed over time from their original source to indoor air, dust, and surfaces. House dust and indoor air samples were collected in 50 California homes: 40 homes in Richmond, CA and 10 homes in Bolinas, CA. Seventy-six SVOCs were analyzed in both air and dust: 24 polycyclic aromatic hydrocarbons (PAHs), 3 polychlorinated biphenyls (PCBs), 2 polybrominated diphenyl ethers (PBDEs), 36 pesticides and 11 phthalates. Overall, we detected 40 target analytes in both house dust and indoor air above method reporting limits. Empirical data were used to explore theoretical partitioning relationships between air and dust to evaluate whether gas-phase air concentrations can be used to predict house dust concentrations. Specifically, gas-phase only concentrations were estimated from total air (particulate and gas phase) concentrations, assuming equilibrium and using residence-specific particulate concentrations. Correlation

analyses revealed significant associations between gas-phase air and dust concentrations for several phthalates, pesticides and PBDE 47. Predicted dust concentrations, which were estimated using gas-phase air concentrations, fraction of organic matter, octanol-air partition coefficient, and dust density, and measured dust concentrations were strongly correlated ( $R^2 \sim 0.7$ ). Understanding partitioning relationships between air and dust will advance our understanding of exposure pathways in the indoor environment; can be used to prioritize exposure measures in large-scale epidemiological studies; and can be used in conjunction with simple, consistent exposure measurement methods, such as a passive air sampler, to fully characterize the indoor environment.

**75 Air-Solid and Solid-Solid Partitioning of Phthalates in the Indoor Environment** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering; H. Kim, Univ of California, Civil and Environmental Engineering. Indoor air and floor dust are severely contaminated by phthalates, which are added to variety of household consumer products such as plastic floor mats, wall papers, carpets, and furniture. Indoor exposure to phthalates has been linked to many adverse health effects such as respiratory disease (e.g., asthma, wheezing) and atopic dermatitis. To investigate how phthalates in PVC based plastic floor mats are transferred to indoor air and floor dust, two different chamber studies were conducted. A piece of vinyl flooring mat was placed inside of a metal chamber, which was maintained at 20 °C. Volatilized phthalates were ventilated with continuous flow of nitrogen gas and captured using activated carbon columns, which were replaced regularly for time-series measurement. As expected, gas phase release of diethylphthalate and dibutylphthalate was significantly higher than the release of diethylhexylphthalate. Gas-phase concentrations of diethylphthalate and dibutylphthalate increased quickly so it is recommended to ventilate indoor air more frequently to minimize indoor exposure to these more volatile phthalates through inhalation. To compare the transfer of phthalates from mats to dust through solid-air-solid partitioning and solid-solid partitioning, sieved outdoor soil particles were placed on the top of a piece of mat and a piece of aluminum foil placed in a closed metal chamber 24 hours without ventilation. Almost all diethylhexylphthalate was transferred to soil particles through direct contact and gas phase transfer was negligible. In the case of diethylhexylphthalate, more frequent floor vacuuming is recommended to reduce the exposure through dust ingestion.

**76 Impact of Plants on Volatile Organic Compounds (VOCs) in Indoor Environments** W.J. Doucette, Utah State Univ, Utah Water Research Laboratory; J. Chard, Utah State Univ. Concerns about the potential build up of volatile organic compounds (VOCs) in indoor air first increased as energy conservation methods minimized the introduction of outdoor air. VOCs including many with documented short- and long-term adverse health effects, are emitted by a wide array of products including: paints, paint strippers, fuels, cleaning supplies, pesticides, building materials, office equipment, and adhesives. These products can release VOCs during use and to lesser extent, when they are stored. Indoor air concentrations of VOCs vary widely, depending on construction materials and specific consumer products within the building but concentrations of most VOCs are consistently higher indoors than outdoors. The use of ornamental plants has been suggested as a simple, unobtrusive, aesthetically pleasing, and cost effective method for sampling and purifying indoor air. The waxy surface of the leaves has the potential to provide a good surface for the passive capture of VOCs. However, the efficiency of capture has not been well characterized and plants themselves give off VOCs. In addition, damp soils can provide an environment for the growth of microorganisms that can impact allergic individuals. By actively drawing air through the root-zone while providing sufficient water and nutrients, a simple bioreactor can be created that can potentially remove VOCs in indoor air through sorption and/or aerobic biodegradation while significantly reducing the potential for allergen production. A flow-through glass and stainless plant growth chamber was constructed to evaluate VOC removal. Potted plants, non-planted controls or plant tissues were placed inside the chamber while one or more representative VOCs found in indoor environments were introduced at environmentally relevant concentrations. Mass balance calculations were performed by comparing the measured concentration of chemical introduced into the chamber to that existing the chamber. In addition, the ability of plants to act as passive samplers was evaluated and compared to conventional passive and active sampling methods.

**77 The Measured Relationship Between Aggregate Material Emission Rates and Building Ventilation Characteristics for Indoor Pollutants** R. Maddalena, Lawrence Berkeley National Laboratory, Department of Indoor Environment; F. Micallef, Lawrence Berkeley National Laboratory and University of Malta, Department of Physics; N. Aquilina, University of Malta, Department of Physics; T. McKone, University of California, Berkeley, University of California and Lawrence Berkeley National Laboratory. Concerns about the potential build up of volatile organic compounds (VOCs) in indoor air first increased as energy conservation methods minimized the introduction of outdoor air. VOCs including many with documented short- and long-term adverse health effects, are emitted by a wide array of products including: paints, paint strippers, fuels, cleaning supplies, pesticides, building materials, office equipment, and adhesives. These products can release VOCs during use and to lesser extent, when they are stored. Indoor air concentrations of VOCs vary widely, depending on construction materials and specific consumer products within the building but concentrations of most VOCs are consistently higher indoors than outdoors. The use of ornamental plants has been suggested as a simple, unobtrusive, aesthetically pleasing, and cost effective method for sampling and purifying indoor air. The waxy surface of the leaves has the potential to provide a good surface for the passive capture of VOCs. However, the efficiency of capture has not been well characterized and plants themselves give off VOCs. In addition, damp soils can provide an environment for the growth of microorganisms that can impact allergic individuals. By actively drawing air through the root-zone while providing sufficient water and nutrients, a simple bioreactor can be created that can potentially remove VOCs in indoor air through sorption and/or aerobic biodegradation while significantly reducing the potential for allergen production. A flow-through glass and stainless plant growth chamber was constructed to evaluate VOC removal. Potted plants, non-planted controls or plant tissues were placed inside the chamber while one or more representative VOCs found in indoor environments were introduced at environmentally relevant concentrations. Mass balance calculations were performed by comparing the measured concentration of chemical introduced into the chamber to that existing the chamber. In addition, the ability of plants to act as passive samplers was evaluated and compared to conventional passive and active sampling methods.

**77.5 Anniston PCB Site: Implications of Field and Laboratory Bioaccumulation Results to an Ecological Risk Assessment** B. Anthony, ARCADIS, Blasland Bouck and Lee; J. Meyer, ARCADIS US, Inc.; D. Ludwig, H. Douglas, M. Shivell, A. Fowler, ARCADIS. Choccolocco Creek and the associated floodplain are part of the Anniston PCB Site (the Site) located in portions of Calhoun and Talladega counties, near Anniston, Alabama. The creek and floodplain are part of Operable Unit 4 (OU-4) for the Site and include over 5,000 acres that line a 35-mile long portion of Choccolocco Creek. PCB concentrations were measured in benthic invertebrate tissue and co-located sediment from nine locations within Choccolocco Creek and three reference locations. Additionally, bioaccumulation of PCBs from 12 Site sediments, two reference sediments, and a laboratory control sediment was investigated in the laboratory through 28-day exposures of a freshwater oligochaete worm (*Lumbriculus variegatus*) to whole sediment. The laboratory bioaccumulation tests were performed in two phases by the US Army Corps of Engineers, in basic accordance with American Society for Testing and Materials (ASTM) and US Environmental Protection Agency (USEPA) guidance using sediment collected from various locations in the creek. Relationships between PCB concentration in sediment and tissue were evaluated in both the field-derived biota and sediment data and in the laboratory bioaccumulation studies. The results of the field measurements in benthic invertebrate tissues and laboratory studies will be used to estimate benthic invertebrate tissue concentrations in the ecological risk assessment foodweb models for OU-4. The predicted tissue concentrations from both approaches and the associated uncertainties will be discussed.

**78 Anniston PCB Site: Implications of Sediment Toxicity-Testing Results to an Ecological Risk Assessment** J. Meyer, ARCADIS US, Inc.; B. Anthony, ARCADIS, Blasland Bouck and Lee; D. Ludwig, H. Douglas, A. Fowler, ARCADIS. Choccolocco Creek and its floodplain are part of the Anniston PCB Site (the Site) located in portions of Calhoun and Talladega counties, near Anniston, Alabama. The creek and floodplain are part of Operable Unit 4 (OU-4) for the Site and includes over 5,000 acres that line a 35-mile long portion of Choccolocco Creek. Chronic toxicity tests were conducted on 26 sediment samples (20 from the Site and 6 from a reference

area), together spanning a wide range of combinations of total PCBs and total organic carbon concentrations and a single laboratory control sediment. Test organisms were a freshwater amphipod (*Hyalella azteca*; 42-d tests) and a freshwater midge (*Chironomus dilutus*; life-cycle tests). The tests were performed by the US Geological Survey and the US Army Corps of Engineers, in basic accordance with American Society for Testing and Materials (ASTM) and US Environmental Protection Agency (USEPA) guidance. The sediments were selected to develop a concentration response relationship based on organic carbon normalized PCB concentrations. Threshold-effects concentrations of PCBs were calculated from the concentration-response relationships for survival, growth, and reproduction endpoints, and were compared to threshold effects concentrations (TECs) and probable effect concentrations (PECs) in published "consensus-based" sediment quality guidelines for freshwater systems. Concentrations of other chemicals were also evaluated to help explain observed sediment toxicity. From those results, the most sensitive of the traditional endpoints will be identified and used to evaluate ecological risk associated with OU-4 sediments. Uncertainties associated with using Site-specific sediment toxicity testing results versus published TECs and PECs will also be discussed.

**79 Overview of the Anniston PCB Site: History and Remedial Investigation** P. Scully, USEPA, Region 4 Superfund; E.g., Macolly, Solutia Inc., Remedial Projects; M. Greenberg, USEPA, Environmental Response Team; A. Fowler, ARCADIS; S. Thoms, USEPA, Region 4 Superfund. The Anniston PCB site (the Site) is located in northeast Alabama. Polychlorinated biphenyls (PCBs) were produced at the Solutia Anniston Facility from 1929 until 1971. The environmental management of the Site is being addressed through a CERCLA Remedial Investigation/Feasibility Study process for three operable units (OUs) that extend over approximately 40 stream miles and 6,000 acres of floodplain. Soil, ground water, and air sampling were conducted to assess risks to human health at the Facility (OU-3). Soil, ground water, sediment, surface water, air and biota data are being used to assess risks to human health and ecological receptors in the residential and non-residential (largely commercial) portions (combined OU-1/OU-2) and less developed, downstream areas (OU-4) of the Site. The floodplain of OU-4 is predominantly comprised of agricultural and forested lands and includes approximately 5,000 acres that surround 35 miles of creek. The range of pathways and the large study area introduce unique challenges within both the human health and ecological risk assessments. The remedial investigation for OU-4 has used a characterization area/exposure unit (CA/EU) approach to streamline the floodplain soil characterization and risk assessment processes. Sediment toxicity and bioaccumulation testing for sediments have been performed to assist in risk management decisions for the Site. This presentation will cover these challenges and will summarize and discuss highlights of the remedial investigation.

**80 Results of a One-Year Fish Consumption Survey in Alabama** J. Schell, Exponent; E. Ebert, Integral Consulting; J. Loper, The Loper Group; N. Wilson, M. Wacksman, A. Fowler, ARCADIS. To support a human health risk assessment for Operable Unit 4 (OU-4) of the Anniston PCB Site, a one-year intercept survey along Choccolocco and Snow Creeks was conducted. The survey collected site-specific information about the extent of usage of the creeks for fishing, locations fished, species and sizes of fish harvested for consumption, methods used to prepare and cook them, rates at which they were consumed, and demographic characteristics of the user population. The survey used interview and "weighting" approaches demonstrated to be effective at other locales. The survey was conducted on 101 survey days, representing 27% of available days during the one-year period. The sampling days were selected based on a stratified random sample method that accounted for season, time of week, location and time of day. In total, only 72 anglers were observed during the one-year survey period and 52 of those individuals completed interviews. After correcting for avidity bias, it was estimated that a total population of 173 anglers fish the OU-4 portions of the creek each year. The interviewed anglers fished an average of four months during the year and took an average of two fishing trips during a typical four-week period. Estimated trips per year ranged from 1 to 54 with an average rate of seven trips per year. Based on the interviews conducted, anglers were successful at harvesting fish for consumption on 15% of the fishing trips taken and only three of the 52 anglers interviewed at the end of their trip had harvested fish for consumption. Consumption rates, which were estimated for each angler who had harvested fish, were based on the mass of fish harvested by trip end, an edible portion of 30%,

the reported frequency of fishing trips, the average success rate, and the number of persons to share in consumption. Currently, a fish consumption advisory is in effect for the creek. The estimated annualized average fish consumption rates estimated from this study ranged from 0.14 g/day to 7.9 g/day with a mean rate of 2.9 g/day. These estimated consumption rates were consistent with those reported for a similar small water body in Alabama that had no advisory at the time of the survey, indicating that suppression of resource utilization is not occurring.

**81 Anniston PCB Site: Human Health Risk Assessment Status** T. Woods, F. Sevard, J. Walsh, Avatar Environmental, LLC; K. Koporec, P. Scully, USEPA Region 4. The human health risk assessment for the operable unit 4 (OU-4) area of the Anniston PCB Site in Anniston, AL, is being conducted. OU-4, the largest of the OUs associated with the Site, encompasses the length of Choccolocco Creek and its floodplain from the confluence with Snow Creek, including the backwater area and upstream on Snow Creek to Highway 78, to Lake Logan Martin. PCBs are the primary Site contaminant. Over the years, the Choccolocco Creek and its floodplain have become contaminated as a result of the operations that have occurred at the Solutia, Inc. plant upstream in Anniston, where PCBs were produced from 1929 through 1971. Environmental investigations have been performed at the plant and its surroundings for the past 30 years. The affected media in OU4 include floodplain soil, creek sediment, fish, and possibly agricultural products grown or raised in the floodplain. The objective of the HHRA is to estimate the health risks as a result of actual or hypothetical contact with the affected media. Likely routes of exposure include incidental soil ingestion, dermal contact with soil and contaminant absorption, ingestion of fish caught from the Choccolocco Creek, and ingestion of agricultural products. The analytical data used in the HHRA were collected in 2007 and 2009 for the purpose of the HHRA and reflect the best estimation of current contaminant concentrations. Based on the current contaminant levels and assumptions on the types of activities that could result in exposure, ingestion of fish caught in the Choccolocco Creek appears to be the exposure pathway that would result in the highest levels of health risk. Even though there has been a 'do not consume' advisory in place for fish in this stream for many years, EPA requires that site HHRA be conducted without consideration of induced restrictions on human exposures. The contamination levels in the floodplain do not appear to result in elevated risk levels assuming a recreational exposure scenario. Potential consumption of fruits, vegetables, and/or livestock grown/raised in floodplain soils could be a health risk concern pending the implementation of the proposed conservation corridor. The HHRA will be re-visited once the status of the conservation corridor is clearly understood.

**82 Anniston PCB Site: Problem Formulation for the Baseline Ecological Risk Assessment along Choccolocco Creek** M.S. Greenberg, USEPA, Environmental Response Team; S.R. Thoms, USEPA, Region 4, Superfund Division, Waste Division, Office of Technical Serv, Superfund Support Branch; D. MacDonald, MacDonald Environmental Sciences Ltd.; D. Charters, USEPA, Environmental Response Team; C.G. Ingersoll, USGS, Columbia Environmental Research Center; P.L. Scully, USEPA; W. Lorentz, USACE; M. Huston, USFWS; A. Fowler, D. Ludwig, ARCADIS. The Anniston PCB site is located in northeast Alabama and the CERCLA Remedial Investigation/Feasibility Study is being conducted in multiple phases across three operable units (OUs) including the facility (OU-3), residential and non-residential (predominantly commercial) areas (OU-1/OU-2), and Choccolocco Creek and its floodplains (OU-4). The screening level ecological risk assessments (SLERAs) were completed for OUs-1/2/3 and OU-4 in 2005 and 2006, respectively. Polychlorinated biphenyls (PCBs) were identified as the primary contaminant of potential concern (COPC) for ecological receptors. Several metals were identified as being present above ecological screening thresholds used in the SLERA and other hazardous substances were also identified as potentially contributing to ecological risk. The results of the OU-4 SLERA and Phase 1 OU-4 investigations were used to develop the problem formulation of the baseline ecological risk assessment (BERA) and an adaptive management process for COPCs in the OU-4 portion of the Site. The baseline problem formulation (BPF) covers the terrestrial, riparian, and aquatic habitats of OU-4. Key ecological receptors were identified from these habitats to develop assessment and measurement endpoints for decision making at the site (including the in-creek portions of OU-1/OU-2). The assessment endpoints include terrestrial and aquatic plant communities, terrestrial and benthic invertebrate communities, fish

communities, birds, mammals, amphibians, and reptiles. Measurement endpoints include environmental sampling, toxicity testing, and exposure modeling to evaluate effects on survival, growth, and reproduction. This presentation will summarize the environmental data from completed investigations on OU-1/OU-2 and OU-3, Phase 1 OU-4 sampling, the results of the SLERAs, and how this information was collectively used to develop the BPF for Choccolocco Creek. A status update for the nearly completed Phase 2 investigations will also be provided.

**83 Childhood Exposure to PCBs from Dietary Supplements Containing Fish Oils** J. Ashley, Philadelphia Univ, Institute for Textile and Apparel Product Safety; J. Ward, ICQ – USA; C. Anderson, Philadelphia Univ, School of Science and Health; M. Schafer, L. Zaoudeh, D. Velinsky, Academy of Natural Sciences. Children's dietary supplements containing fish oils have become a popular means of increasing children's intake of polyunsaturated fatty acids (PUFAs). However, there is growing concern that the levels and potential health effects of lipophilic organic contaminants such as polychlorinated biphenyls (PCBs) may diminish some of the health benefits associated with daily consumption of fish oil supplements. In this study, thirteen over-the-counter children's fish oil supplements were analyzed for PCBs and daily exposures were calculated. Concentrations of t-PCBs ranged from 0.4 to 20.0 ng/g wet wtg and were low compared to our recent study of adult fish oil supplements (3.1 to 264 ng/g wet wtg). Based on manufacturers' recommended dosages, a child's daily intake of PCBs would range from 2.8 to 50.3 ng/day. Daily consumption of fish oil supplements expose children to PCBs, albeit at low levels. In comparison to ingestion of fresh or frozen fish to gain equivalent amounts of PUFAs contained in supplements, children's fish oil dietary products may decrease daily PCB exposure.

**84 Choccolocco Creek Conservation Corridor – Watershed Protection Through Proactive and Permanent Institutional Controls** E.G. Macolly, Solutia Inc., Remedial Projects; J. Loper, The Loper Group; M. Shivel, A. Fowler, ARCADIS. Choccolocco Creek and its floodplain are part of Operable Unit 4 (OU-4) of the Anniston PCB Site located in Calhoun and Talladega counties in Alabama. The floodplain includes over 5,000 acres adjacent to 35 miles of Choccolocco Creek. This presentation describes the development and implementation of a Conservation Corridor including planning and design activities, challenges associated with implementation, current status and the benefits of a watershed-based protection project in the long-term remedial resolution of a complex floodplain site. The Conservation Corridor was designed using a GIS database including tax parcel information and years of investigation data. The GIS was used to evaluate the extent of the Corridor, identify land parcels essential to the success of the program, and prioritize the parcels to maximize watershed protection in the shortest timeframe. Tax parcels were ranked based on distance downstream from the Anniston/Oxford area, Creek frontage and acreage within the Corridor footprint. The resulting Conservation Corridor is implementable, protective of the undeveloped nature of the Creek and floodplain, protective of the existing land uses and landowner rights in perpetuity. The Conservation Corridor is being implemented in a phased approach through a non-profit land trust that manages over 150,000 acres of conservation land in Georgia and Alabama. The initial phase focuses on a 10 mile section of the floodplain downstream of the urbanized portions of Anniston and Oxford. Much of this first phase (842 of ~1,500 planned acres) is under easement or ownership by the land trust. The land trust hopes to extend the Corridor downstream to the Coosa River and upstream into the Talladega National Forest. While the Conservation Corridor is a standalone project implemented to protect the Creek and its riparian floodplain, there are many additional benefits. These include increased educational and recreational opportunities and a role in the selection of a remedial solution. The Corridor also acts as a focal point for local land owners and other stakeholders to drive a range of watershed improvement projects. By limiting future development adjacent to the Creek and keeping farm animals from entering the Creek, the Corridor is a direct mechanism for improving habitat and water quality. The Conservation Corridor is also a long-term land-use institutional control that will be an integral part of the selected remedy for OU-4.

**85 Assessing the Bioavailability of Parent and Alkylated-PAHs Using Passive Samplers in Petroleum Oil-Impacted Sediment Treated by Activated Carbon** Y. Choi, Y. Cho, R.G. Luthy, Stanford Univ, Dept of Civil and Environmental Engineering. Alkylated-polycyclic aromatic



hydrocarbons (PAHs) are known to constitute about 90% of total PAHs in petroleum oil. Yet they are seldom studied in petroleum oil-impacted sites because of the difficulty in characterizing the large number of compounds that fall in this category. In this study, we have developed a rigorous analytical technique to characterize 20 different groups of alkylated-PAHs, ranging from C1- to C4-PAHs, as well as 18 individual parent-PAHs, using gas chromatography-mass spectrometry (GC-MS) in a selective ion monitoring (SIM) mode. Polyethylene (PE) passive samplers were employed to enhance the measurability of aqueous concentrations, which is indicative of the bioavailable fraction of the compounds. The analytical procedure we developed has been used to study the reduction in aqueous concentrations of PAHs by activated carbon amendment in a petroleum oil-impacted sediment. The sediment sample had a concentration of 11.6 mg/kg as parent-PAHs and 114 mg/kg as alkylated-PAHs. The amendment of 5% (sediment dry weight basis) activated carbon, 75–300 µm in grain size, reduced the uptake in PE samplers by 82% and 74%, respectively, for parent- and alkylated-PAHs after 8 weeks of thorough mixing. The effect of activated carbon amendment was greatly enhanced by using a finer activated carbon particles. Using an activated carbon grain size of < 32 µm, the reduction of PE uptake increased up to 98.9% for both parent- and alkylated-PAHs. Additional experiments are underway to study the activated carbon performance in long-term tests and to measure the PE-water partitioning coefficients of PAHs that will be used to correlate the PE uptake with aqueous concentrations.

**86 Reducing Bioavailability at the Sediment Water Interface by Active and Passive Thin Capping – Field Testing in Norway** E. Eek, Norwegian Geotechnical Institute, Environmental Technology; A. Oen, Norwegian Geotechnical Institute, Dept Environmental Engineering; G.D. Breedveld, Norwegian Geotechnical Institute, Univ of Oslo, Institute for Geosciences; M. Schaanning, Norwegian Institute for Water Research; G. Cornelissen, Norwegian Geotechnical Institute. In order to study the effectiveness of thin layer capping with active and passive materials in reducing the bioavailability persistent organic pollutants (POP) at the sediment surface, six different test plots have been constructed and subsequently monitored in contaminated Norwegian fjords. As a part of this study both placement success chemical availability (as sediment to water flux) and benthic fauna health where monitored. Here we focus on flux of freely dissolved PAHs, dioxins and TBT from capped and uncapped seabed sediments. Outside the TBT contaminated Fiskerstrand ship yard on the west coast two test plots where constructed: 7 cm thick cap of fine grained limestone slurry and 7 cm thick cap of fine grained limestone slurry mixed with activated carbon (AC). In the dioxin contaminated Grenlandfjords on the south east coast of Norway four different test plots where constructed; 5 cm thick crushed limestone cap, 5 cm clean clay cap, 2 cm cap with clean clay mixed with AC at two different water depths 30 m and 100 m. On each of these plots three infinite sink benthic flux chambers where deployed to monitor the release of POPs from the seabed to the overlying water. The infinite sink benthic flux chambers work by enclosing a volume of water and an area of the seabed and collects POPs on an infinite sink material (triolin filled Polyethylene membranes or silicon rubber sheets) inside the chamber. By extracting and measuring the amount of POPs collected on the infinite sink the flux can be calculated from the enclosed seabed area and the deployment time (Eek et al. 2010 ES&T Vol. 44). The measured flux, within a year after capping, was lower on all capped test plots compared to flux from the test plots before capping or from adjacent uncapped reference fields, and showed that the flux was reduced by 75 – 99 % by a passive capping layer of 5 cm or more. However, at the fields with 2 cm AC containing cap, only a 55 – 63 % reduction was found. The greater influence of bioturbation in the thinner capping layers and the slow mass transference of POPs from sediment particles to AC particles is a likely explanation to these observations.

**87 Bioaccumulation of Highly Hydrophobic Organohalogen Flame Retardants in Sediment to the Oligochaete, *Lumbriculus variegatus*** B. Zhang, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; J. You, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry. Recent studies reported that highly hydrophobic organohalogen flame retardants, such as decabromodiphenyl ether, decabromodiphenyl ethane, and dechlorane plus, which had log Kow values greater than 9, were ubiquitous in the environment. Due to their hydrophobicity, organohalogen flame retardants tended to be associated to sediment and high concentrations of

these contaminants have been widely detected in sediment in the Pearl River Delta (PRD), South China. However, studies on their bioavailability to benthic organisms were limited. In the current study, bioavailability of the three target halogenated flame retardants was assessed by exposing the oligochaete, *Lumbriculus variegatus* to both spiked and field-contaminated sediments. The spiked sediment was dosed in two different concentrations of the target compound while the field sediment was collected from an electronic waste recycling site in the PRD. Meanwhile, the freely dissolved chemical concentrations in sediment porewater were also measured using matrix solid-phase microextraction. The uptake and depuration curves in the bioaccumulation tests showed that these highly hydrophobic contaminants were bioavailable to the worms. On the other hand, the freely dissolved concentrations of the contaminants in porewater were very low indicating ingestion of sediment particles may be the major uptake routes for these highly hydrophobic organohalogen flame retardants.

**88 Use of SPME Fiber and Tenax Methods to Predict the Toxicity of Pyrethroids in Field Sediments** A.D. Harwood, Southern Illinois Univ, Zoology; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology; D. Weston, Univ of California, Dept of Integrative Biology. The presence of pyrethroids in both urban and agricultural sediments at levels lethal to invertebrates has been well documented. However, variations in bioavailability among sediments make accurate predictions of toxicity based on whole sediment concentrations difficult. A proposed solution to this problem is the use of bioavailability-based estimates such as SPME fibers and Tenax beads. Previous research has demonstrated that both methods serve as accurate predictors of bioavailability and mortality. This study compared three methods to predict bioavailability and ultimately toxicity: field-deployed SPME fibers, laboratory-exposed SPME fibers, and a 24 hour Tenax extraction. The objective was to compare the ability of these methods to predict the toxicity of pyrethroid contaminated field sediment to three benthic species *Hyalella azteca*, *Chironomus dilutus*, and *Hexagenia* sp. Six sites in central California with known pyrethroid contamination were chosen. At each site, six Plexiglas holders containing SPME fibers encased in copper mesh were deployed and approximately 4 liters of sediment was collected. Standard 10 day toxicity evaluations were conducted on the six sediments for the three species in concurrent separate exposures. In addition, SPME fibers were exposed to sediments, a 24 hour Tenax extraction was performed, and pyrethroid sediment concentrations were determined using standardized laboratory methods. All SPME fibers were exposed for 42 days to ensure they had reached equilibrium. All six sediments were toxic to at least one species. Typically, *H. azteca* were the most sensitive followed by *Hexagenia* and *C. dilutus*. For both Tenax and SPME extracted concentrations a bioavailable toxic unit was calculated (SPME or Tenax concentration/sediment LC50). This value was then correlated to mortality for each method, including standard toxic units for the bulk sediment concentrations. The study demonstrates the applicability of bioavailability-based toxicity estimates versus standard methods.

**89 Bringing (Bio)accessibility Extractions to the Next Level – Combining Mobilisation Medium and Infinite Absorption Sink** V. Gouliarmou, Aarhus Univ, National Environmental Research Institute, Environmental Chemistry and Microbiology; E. Edelmann, A.P. Loibner, Univ of Natural Resources and Applied Life Sciences, Agrobiotechnology – IFA Tulln; C. Collins, Reading Univ, Soil Research Center; P. Mayer, Aarhus Univ, National Environmental Research Institute. Soil bioaccessibility extraction techniques are generally simple dissolution experiments, where the fraction of compounds that is transferred to the medium is measured and considered to be bioaccessible. However, such techniques can lead to an underestimation of bioaccessibility, since they do not account for the consumption of contaminants by either degradation or absorption. It is therefore crucial to develop practical bioaccessibility extraction approaches that combine both mobilisation and consumption processes. The mobilisation medium can be chosen to either maximize desorption without attacking the matrix or even to simulate the relevant organism conditions. A sorbent can act as an infinite diffusion sink for continuously removing the mobilized contaminants from the medium. Initially, we combined cyclodextrin extraction with a polymer of poly(dimethylsiloxane) and activated carbon which lead to the contaminant trap method1. This contaminant trap is a practical and simple approach for the isolation and quantification of the desorption resistant contaminants in soils, its main limitation being that it is not possible to

back extract from the polymer. The next step was to find a polymer material and format that can act as infinite sink and allow simple back extraction. Silicone rods were chosen, which are already used in silicone rod extraction and passive sampling. This resulted in a better and more relevant bioaccessibility extraction approach and simplified the analytical procedures. The silicone rods were applied to both cyclodextrin solutions and artificial digestive fluids, using PAHs as model compounds. Passive dosing<sup>2</sup> was applied to determine the free fraction and partitioning of PAHs in cyclodextrin and digestive fluids, which in turn was used for sink dimensioning. Validation experiments with spiked cyclodextrin and digestive fluids confirmed an efficient trapping of PAHs by the silicone rod. References (1) Mayer, P.; Olsen, J. L.; Gouliarmou, V.; Hasinger, M.; Kendler, R.; Loibner, A. P. *Environmental Science & Technology*, 2011, 45(7), 2932-2937. (2) Birch, H.; Gouliarmou, V.; Lutzhoft, H. C. H.; Mikkelsen, P. S.; Mayer, P. *Analytical Chemistry* 2010, 82, 1142-1146.

**90 Sediment Quality Guidelines in British Columbia, Canada** J. Arblaster, Simon Fraser Univ, Resource and Environmental Management; F.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment); M.G. Ikonomou, Institute of Ocean Sciences. Current methods used to derive Sediment Quality Guidelines (SQG) in Canada do not account for the biomagnification of chemicals in marine food chains, resulting in SQGs that are insufficiently protective of upper trophic level organisms such as marine mammals, birds and humans. The Biota-Sediment Accumulation Factor ( $BSAF = C_{\text{biota}}/C_{\text{sediment}}$ ) quantifies the relationship between concentrations in organisms and the sediment and can be applied in risk assessment or to derive SQGs that by accounting for biomagnification of chemicals are more protective of ecosystems. Here, we investigate the use of BSAF for guideline development in B.C. using empirical data and bioaccumulation modeling. PCB data for four sites on the B.C. coast was used to derive BSAFs, assess how protective of upper trophic level populations current guidelines are and to derive new SQG. A west coast bioaccumulation model was developed for additional species and human health risk assessment. Empirical BSAF values are highest for Dungeness Crabs and Harbor Seals, but are highly variable both within and between sites. Species with similar trophic position show similar BSAF values indicating that species-specific traits do not play a significant role in BSAFs. The model shows good agreement with empirical data in Vancouver and the Strait of Georgia, but less so in Victoria and the Central Coast where BSAFs are lower. Applying empirical and model derived BSAF values for risk assessment shows that current guidelines fail to protect the majority of Orca whale, Stellar sea lion and harbor seal populations based on toxicological reference values. Current guidelines also do not ensure fish and shellfish populations are below Tissue Residue Guidelines for human consumption. SQGs back-calculated from relevant endpoints for human health, marine mammal population health and to meet Chinook salmon tissue residue guidelines ranged from 0.002 µg/kg dw to 2.7 µg/kg dw – substantially lower than the current B.C. SQG of 20 µg/kg dw.

**91 Improving Predictability of Sediment-Porewater Partitioning Models using Trends Observed with PCB-Contaminated Field Sediments** S.B. Hawthorne, Univ of North Dakota, Energy and Environmental Research Ctr; D.J. Miller, C.G. Grabanski, Univ of North Dakota, Energy and Environmental Research Center; H.H. Arp, Norwegian Geotechnical Institute, Environmental Engineering. More than 1900 sediment-water partitioning coefficients were measured for 58 polychlorinated biphenyl (PCB) congeners in 53 historically-contaminated sediments collected from 10 urban and rural waterways in the United States and Canada. Freely-dissolved porewater concentrations were determined using passive sampling with polyoxymethylene. Measured total organic carbon (TOC)/water partitioning coefficients, KTOC, ranged from one to nearly three orders-of-magnitude higher than typical literature values based on spiking experiments and model predictions. Although total PCB concentrations ranged from 0.08 to 194 mg/kg, the more highly-contaminated sediments showed only slightly lower KTOC values than less-contaminated sediments. No correlation was observed between log KTOC values and sediment TOC, black carbon (BC), or BC/TOC fractions ( $r^2$  typically < 0.1). Utilizing a two-carbon model incorporating anthropogenic BC did not improve predictions over a one-carbon TOC model. A comparison of models recently validated for historically contaminated sediments showed that a coal-tar poly-parameter linear-free energy relationship (PP-LFER) and the Raoult's Law model were successful at predicting average log KTOC values, without the need for any calibration

or fitting (within a factor of 10 more than 90 % of the time, and within a factor of 30 more than 99 % of the time). Predictions were further improved by the introduction of a Weathering Factor (WF) that accounts for the relative depletion of lower molecular weight congeners due to weathering processes (e.g., desorption, degradation). Highly weathered sediments (with a WF near 1) tended to follow the coal-tar PP-LFER and Raoult's Law model the closest. Fresher sediments (with WF

**92 Modeling trade-offs between pollutant sorption and biodegradation in biochar-amended soils** D. Werner, Newcastle Univ, Civil Eng. and Geosciences; K.M. Bushnaf, Newcastle Univ; S. Puricelli, S. Saponaro, Politecnico di Milano. Biochar addition to soil is being investigated as a novel technology to remediate polluted sites. A critical consideration is the impact of biochar on the intrinsic microbial pollutant degradation, in particular at sites polluted with a mixture of readily biodegradable and more persistent organic pollutants. Pollutants bound in the micropores of carbonaceous sorbents such as biochar or activated carbon become less accessible for bio-uptake by soil organisms, bacteria or plants, and the addition of activated carbons or biochars thereby reduces the transfer of these pollutants from soil into the terrestrial food-chain. This occlusion may, however, also reduce the pollutant's availability for microbial break-down and increase persistence. On the other hand, biochar addition to degraded soil has been shown to improve the soil structure and fertility and microbial cell numbers. Because of these antagonistic effects, the effect of biochar on the microbial degradation of soil pollutants cannot be readily anticipated. We therefore studied the impact of biochar (2% on dry weight basis) on the volatile petroleum hydrocarbon intrinsic biodegradation in an aerobic sandy soil with batch and column studies. The soil-water partitioning was enhanced in the biochar-amended soil and petroleum hydrocarbon vapour migration was retarded accordingly. The  $K_d$  value of toluene increased 36 times, because of the ability of this compound to interact via  $\pi$ - $\pi$  electron forces with the aromatic surface of the biochar. Additional methyl-groups on the aromatic ring appear to interfere with these interatomic interactions, since m-xylene and 1,2,4-TMB did not show a comparable enhancement in their  $K_d$  value in the biochar-amended soil. We employed a numerical pollutant fate model to interpret measured VOC concentration profiles in soil with and without biochar amendments at various distances from a NAPL source. The column study indicated similar total petroleum hydrocarbon degradation in soil with and without biochar, greater volatilisation of all petroleum hydrocarbons from soil without biochar and greater biodegradation of linear, branched and cyclic alkanes in soil with biochar. We conclude that the total petroleum hydrocarbon degradation rate was controlled by a factor other than substrate availability in the soil we investigated, and the reduced availability of monoaromatic hydrocarbons in biochar amended soil led to greater biodegradation of the other petroleum compounds.

**93 Relationship Between the BCF, BAF and the TMF** E.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment); A.R. Brisebois, Simon Fraser Univ, Resource and Environmental Management; H.A. Leslie, Institute for Environmental Studies, VU Univ; P. Leonards, Institute for Environmental Studies, VU Univ, Chemistry and Biology, VU Univ, Institute for Environmental Studies. There is a reasonable degree of consensus among bioaccumulation scientists on the use of Trophic Magnification Factors (TMF) as "conclusive" evidence of the bioaccumulative nature of chemicals in the environment. However, regulatory criteria for the determination of whether a substance is bioaccumulative mostly rely on the use of the Bioconcentration Factors (BCF), measured in laboratory tests. In this paper, we present the results from laboratory, field and modeling studies aimed at testing the hypothesis whether the BCF is a good predictor of the TMF. Our studies involved (i) the compilation of BCFs for a range of potentially bioaccumulative substances through literature studies; (ii) a field study of the food-web bioaccumulation of the same substances in Western Scheldt Estuarine food-web aimed at determining the TMF in food-webs containing both water and air-breathing organisms; (iii) a modeling study aimed at formulating and exploring the relationship between the BCF and the TMF. We conclude that the BCF can be a useful predictor of the TMF for a range of chemicals with specific characteristics and under specific ecological conditions. We also conclude that there are two major types of "exceptions" where the BCF does not provide accurate information about the bioaccumulative nature of chemicals in the environment. The first exception deals with substances that show a relatively low BCF but have a high TMF. The second exception is for chemicals that

exhibit a high BCF in laboratory tests, but which do not show biomagnification in food-webs and have a low TME. Supported by model calculations, we propose a set of simple rules that may be useful in the interpretation of laboratory based bioconcentration factors in terms of their bioaccumulative nature in the environment. These rules may be helpful to regulators involved in bioaccumulation assessments and to scientists that apply bioconcentration studies to study the bioaccumulative nature of chemicals in the environment.

**95 In Situ Mercury Uptake by Aquatic Invertebrates in the South River, Virginia** J.R. Flanders, W.J. Reese, G. Murphy, URS Corporation; R.G. Stahl, DuPont Company, Corporate Remediation Dept, Principal Consultant. The relative importance of mercury uptake through aqueous and dietary pathways is critical to understanding the movement and biomagnification of mercury from physical media and primary producers to aquatic consumers. Aqueous and dietary uptake of inorganic mercury (IHg) and methylmercury (MeHg) by aquatic invertebrates were assessed along a gradient of mercury concentrations in physical media (surface water and sediment) in the South River, Virginia. The study design included a seven-day uptake period for mayfly nymphs (i.e., *Maccaffertium* sp.) and crayfish (i.e., *Orconectes* sp.) transplanted from a reference river and deployed in microcosm chambers containing cobble, pebble, and gravel substrates. The dietary treatment contained substrates colonized with native epiphytic communities to provide food resources for study organisms, while the aqueous treatment contained uncolonized substrates. The microcosm chambers were designed to facilitate surface water and pore water exchange. Co-located measurements of IHg and MeHg in physical media and primary producers were collected on day-0 and day-7 of the study period. In addition, resident mayfly nymphs and crayfish were collected on day-0. The experiment was performed in spring and summer to determine seasonal effects on uptake. Preliminary results indicate that uptake of both IHg and MeHg by mayfly nymphs were significantly greater than by crayfish for both seasons, treatments, and study sites. Furthermore, uptake of IHg and MeHg by mayfly nymphs did not differ consistently among seasons or uptake pathways. However there were significant differences between study sites.

**96 TVA Kingston Ash Spill: A Preliminary Weight-of-Evidence for Aerial Insectivores Exposed to Selenium and Other Metals** S. Young, T. Schlekert, D. Jones, A.R. Stojak, ARCADIS; T.H. Henry, Tennessee Valley Authority; N.E. Carriker, TVA Kingston Ash Recovery Project, Kingston Fly Ash Recovery Project. The December 2008 Kingston ash spill spawned predictions of dire effects on the aquatic ecology in the region as a result of bioaccumulation of ash-related constituents. Tennessee Valley Authority (TVA) proactively developed a comprehensive and integrated research program to evaluate the potential for adverse environmental effects of the spill. One aspect of that work focused on accumulation and effects of ash-related constituents on aerial-feeding insectivores consuming emergent aquatic insects from the Kingston river system. This consisted of assessing potential risks to aerial-feeding insectivorous birds and mammals, as represented by the tree swallow (*Tachycineta bicolor*) and gray bat (*Myotis grisescens*). This talk focuses on the assessment for tree swallows, which have been shown to be a useful indicator of bioaccumulative constituent exposures. Tree swallows feed on emergent aquatic insects whose larval forms may accumulate constituents from submerged sediments. Thus, tree swallows may exhibit adverse effects of constituents transferred from the sediment to terrestrial systems. Three types of data are available for tree swallows: dietary exposure models, measured body burdens, and population survey results. The food web model used a combination of standard and site-specific factors to estimate conservative and realistic dietary exposures. Notable is the use of measured metal concentrations in adult mayflies (*Hexagenia bilineata*). Body burden samples and field survey data were obtained from nest boxes in the vicinity of the spill and at reference reservoirs. Several tree swallow colonies were established in 2009 and 2010 and evaluated for exposure and reproductive productivity. Nests were monitored regularly and accessed multiple times to obtain egg and nestling samples. Metal and metalloid concentrations were evaluated relative to spill proximity and sediment data as measures of exposure to ash-related constituents. Selenium tissue concentrations were compared with literature data to estimate potential effects. Parameters such as clutch size and hatchling success are direct measures of potential population-level effects. The preliminary weight-of-evidence suggests that although exposure to some ash-related constituents is elevated in the river system, potential adverse effects are not clearly evident. This

presentation highlights key findings and discusses the relative strengths and weaknesses of the various lines of evidence.

**97 Bioaccumulation of PCBs and PBDEs in Marine Zooplankton from the Strait of Georgia, British Columbia, Canada** H. Frouin, N. Dangerfield, Fisheries and Oceans Canada, Institute of Ocean Sciences; R. MacDonald, Fisheries and Oceans Canada, Institute of Ocean Sciences; P. Shaw, Environment Canada, Science Division; P. Ross, Fisheries and Oceans Canada, Institute of Ocean Sciences. The accumulation of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in plankton plays a significant role in the transport and fate of these compounds in aquatic food webs. We collected water samples at three stations in the Strait of Georgia to measure concentrations of 204 PCB and 61 PBDE congeners. Samples were collected at both near surface and near bottom in each of four seasons over a one year period. In an effort to capture movement in and out of the system, a minimum of one tidal cycle was sampled with Infiltrax™ in situ samplers. Zooplankton samples were collected vertically from the water column using a SCOR plankton net with 255 µm cod end. PCBs and PBDEs in water samples were analyzed in two fractions, representing dissolved (XAD) and particle-bound (0.7 µm; GF/F). The spring phytoplankton bloom may explain higher plankton PCB and PBDE concentrations in the June samples. The PCB/PBDE ratios were higher in dissolved phase than in particle-bound phase, this partitioning being partly related to chemical properties of the PCBs and PBDEs. This was particularly evident for the "heavier" BDE-209, which partitioned preferentially onto particles. The PCB pattern was dominated by tri- and tetra-PCB compounds in both dissolved and particle-bound fractions and by penta- and hexa-PCB compounds in zooplankton. BDE-47, -99, -100 and -209 dominated in environmental matrices and zooplankton. Principal components analysis (PCA) further revealed a congener-related pattern difference between zooplankton, dissolved and particle-bound fractions, highlighting the importance of particles in driving fate of organic contaminants in both environmental and biological matrices. The logBAFLWs (Bioaccumulation factor on a lipid weight basis) for PCBs and PBDEs versus octanol-water partition coefficient (logKow) relationship was near 1:1 for POPs within the log Kow range of 5 to 7. A curvilinear model provided a better relationship between these two variables when POP compounds with log Kow > 7 were included, suggesting that POPs ~ log Kow 5 to 7 in zooplankton are at equilibrium with water and that physical partitioning, rather than biotransformation, is the major factor governing POP profiles in marine zooplankton. Given increasing PBDE concentrations over recent decades, and the persistence of PCBs in the environment, this uptake by zooplankton represents an important prelude to further food web-based biomagnification.

**98 Spider Mediated Trophic Transfer of PCBs from Aquatic to Terrestrial Food Webs** D. Delach, Clemson Univ, Environmental Toxicology; C.M. Lee, Clemson Univ, Environmental Engineering and Earth Sciences Dept; D. Walters, USGS, Fort Collins Science Center, Ecologist. Trophic transfer within the Lake Hartwell, Twelve Mile Creek watershed has been documented from the sediment to biofilms to fish, resulting in concentrations above 2ppm, which is the limit established by the FDA for sale of commercial seafood in the US. There is also transfer from the aquatic to the terrestrial components of the ecosystem. Emergent insects that develop in the sediment function as biovectors of contamination when they are captured by riparian spiders. This directs the PCB contamination to predators such as lizards, amphibians and birds. Risk of potentially harmful PCB levels to such predators increases as the spiders constitute higher percentages of the diet and as PCB concentrations increase. Sampling from a variety of spider families has indicated that the enantiomeric fraction of chiral congeners as measured by GC-ECD differs for families and over the contamination gradient. Sediment concentrations of 15ng/g have been measured, resulting in a bioaccumulated concentration of up to 2400ng/g in spiders with enantiomeric fractions (EFs) that differ from their prey sources. For example, *Chironomus dilutus* have racemic EFs (0.50) for PCB congeners 91 and 95, whereas *Tetragnathidae* spiders have significantly nonracemic EFs at 0.28 for PCB 91 and 0.25 for PCB 95. Spiders have yet to be included in trophic transfer studies despite their important role mediating the transfer from aquatic to terrestrial food webs. With a global distribution, terrestrial and aquatic insect prey sources, and unique trophic placement, their potential use in toxicological studies can create opportunities to assess spatial and temporal differences in biotransformation.



**99 Trophic Transfer of Decamethylcyclopentasiloxane (D5) in Aquatic Food Webs in Comparison to Polychlorinated Biphenyl Materials (PCB-153 and PCB-180)** D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101); R.M. Seston, J.A. Durham, K.B. Woodburn, Dow Corning Corporation, Health & Environmental Sciences. The potential of a chemical substance to accumulate in aquatic organisms and to increase in concentration with increasing trophic level are criteria used to classify substances as being persistent, bioaccumulative, and toxic (PBT). Bioaccumulation is the accumulation of a substance in the tissues of a living organism through any route. Biomagnification is the increase in concentration of a substance in living organisms that are separated by a single trophic level step on a food chain. Trophic magnification, which describes the increase in concentration of a substance in living organisms that occupy successively higher trophic levels within a food web, is used to assess bioaccumulation and biomagnification of chemicals in the environment. Sediments and aquatic organisms were analyzed to evaluate trophic magnification of decamethylcyclopentasiloxane (D5; CAS No. 541-02-6) in the freshwater food web of Lake Pepin (Minnesota, USA) and in the marine food web of Oslofjord (Norway). Isotopic signatures for the stable isotope of nitrogen ( $^{15}\text{N}$ ) and carbon ( $^{13}\text{C}$ ) were used to identify trophic level positions occupied by the organisms and to evaluate the flow of carbon in the food webs. Additional samples from Lake Pepin were also analyzed for the polychlorinated biphenyl materials 2,2',4,4',5,5'-hexachloro-1,1'-biphenyl (PCB-153; CAS No. 35065-27-1) and 2,2',3,4,4',5,5'-heptachloro-1,1'-biphenyl (PCB-180; CAS No. 35065-29-3), which are "legacy" chemicals that are both known to bioaccumulate in aquatic organisms and biomagnify in aquatic food webs. Concentrations of D5 in Lake Pepin and Oslofjord were greatest in the lowest trophic levels and significantly decreased up the food web, with the lowest concentrations being observed in the highest trophic levels. In contrast, concentrations of PCB-153 and PCB-180 in Lake Pepin were lowest in the lowest trophic levels and highest in the highest trophic levels. These results indicated that trophic dilution of D5, not biomagnification, was occurring across the aquatic food webs. The high level of agreement for results between Lake Pepin and Oslofjord demonstrated that trophic dilution of D5 was not related to differences in the environment (freshwater vs marine) or to exposure.

**100 Trophodynamics of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Coastal Waters Food Web from Surabaya City, Indonesia** M. Ilyas, Ehime Univ, Center for Marine Environmental Studies (CMES), Environmental Chemistry; A. Sudaryanto, I.E. Setiawan, Agency for the Assessment & Application of Technology, Technology Center for Marine Survey; A.S. Riady, Ehime Univ, Center for Marine Environmental Studies (CMES), Environmental Chemistry; C.K. Hyeon, Kyung Hee Univ, Environmental Science and Engineering; T. Isobe, Ehime Univ, Senior Research Fellow Center; S. Takahashi, Ehime Univ; S. Tanabe, Ehime Univ, CMES, Environmental Chemistry. Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) was studied in the coastal waters food web from the highly industrialized city of Surabaya, Indonesia. We measured the concentrations of PCBs (62 congeners) and PBDEs (42 congeners) as well as carbon and nitrogen stable isotope ratios in wild fish and shellfish ( $n=33$ ) from Surabaya coastal waters to elucidate the contamination status, trophic positions in food web and trophic magnification factors (TMFs) of the contaminants. Concentration ranges and median of PCBs and PBDEs were 84–1600 (410)  $\text{ng g}^{-1}$  lw and ND–43 (5.6)  $\text{ng g}^{-1}$  lw, respectively. BDE-47 and -209 were the predominant PBDE congeners in most of the samples. BDE-209 was particularly detected in benthic species, indicating the bioaccumulation of BDE-209 associated with sediment. On the other hand, CB-153, -138 and -180 were the predominant congeners of PCBs in fish tissues. Concentrations of PCBs and PBDEs in biota were significantly correlated with lipid contents ( $p < 0.001$ ). The TMFs of PCBs and PBDEs indicated that most of PCBs and PBDEs were not biomagnified in the biota of the present study, including total PCBs, CB-153, -138; total PBDEs, BDE-47, -153 and -209, for which the TMFs values were 0.95, 0.97, 0.91; 0.69, 0.86, 0.89 and 0.65, respectively. However, CB-180 and -170 have TMFs  $> 1$  (1.4 and 1.3), suggesting that these congeners are biomagnified in the coastal water food web of Surabaya. This study reported for the first time the biomagnification characteristics of organic contaminants in coastal ecosystem from Surabaya City, Indonesia.

**101 A Case Study of Regional Ecological Risk Assessment Based on Land Use Analysis in Beijing** G. Xiurui, Beijing Univ of Technology, College of Environmental and Energy Engineering; C. Dongsheng, H. Hongwei, C. Shuiyuan, Beijing Univ of Technology. Ecological Risk Assessment (ERA) has been focused by researchers recently. Regional Ecological Risk Assessment (RERA) could be an effective way to describe and assess the adverse effect to ecosystems from environment pollutions, human activities or nature disasters on regional scale. Its objective is to providing the support for the regional risk management. Generally, RERA can be divided into 4 parts: risk receptor analysis, risk source analysis, exposure and hazard analysis and risk characterization, etc. This paper conducted a RERE study in Beijing. It can be reclassified into 5 categories of representative ecosystems for the 16 kinds of land-use in Beijing as risk receptors, which are forest, lawn, cultivated field, water and urban area. By screening and identifying all the risk sources in Beijing it was determined that drought, air pollution and dust were the main risk sources, for which the spatial distribution, intensity and probability were analyzed. For the exposure and hazard analysis, this paper chose the ecological loss index to reflect the ability of receptors to resist the disturbance. AHP (Analytic Hierarchy Process) was employed to assess the weights of main risk sources. Risk characterization can get the comprehensive ecological risk value for each county in Beijing, according to which 5 levels of ecological risk were divided by ArcGIS software. The results showed that the value of ecological risk ranged from 0.0027-0.0535, with Miyun, Huashou and Yanqing being of highest risk, downtown area being of lowest risk, and Fangshan, Tongzhou and Shunyi being of middle risk. It can be concluded that the risk assessment results lied in the 95% confidence interval by the uncertainty analysis. Finally, some policy suggestions for the ecological management measures for local government were presented.

**102 An Integrated Risk Assessment Framework to Advance Environmental Quality and Species Recovery Initiatives at the Watershed Scale** R. Johnston, US Navy, Marine Environmental Support Office-NW; J. Brandenberger, Battelle Pacific NW Division-MSL. Integrated ecosystem assessments for large ecosystems have a critical need to identify and rank risk drivers to ensure that recovery actions are focused on the most urgent and important problems. Effective ecosystem management and recovery will require a rigorous and holistic assessment of the relative risks by relating sources of stress like toxic loadings, hypoxia/anoxia events, nuisance species, and loss of habitat to the structure and function of the food web and the critical provisioning and sustaining ecological processes needed to obtain ecosystem recovery. To address this need, an integrated risk assessment conceptual model framework is proposed that links the sources of stress (threats and drivers) to secondary stressors (environmental media and structures) and identifies the pathways (pressures and processes) by which primary and food web receptors can be impacted. The framework allows identification of assessment endpoints, measurement endpoints, and available data sources that can be used to assess relative ecological and human health risks. Application of a numerical modeling framework linking watershed scale processes to the food web and cumulative net ecosystem benefit will improve the risk assessment by increasing the capacity to assess risks to higher levels of ecological organization (population and community) and allow spatially explicit characterizations of risk to be developed. Regional or watershed scale approaches are necessary to properly bound and address the complexity of the problem and develop meaningful management actions. The framework encompasses and harmonizes requirements for three of the major regulatory drivers for ecosystem recovery – restoration (ESA), cleanup (CERCLA), and water quality improvement (CWA). By providing an effective feedback between what is causing risk and actions to manage risk, the framework also supports adaptive learning and improves the understanding needed for effective ecosystem management.

**103 Assessing Ecological Risk Over Large Geographic Scales with Spatial Applications of Eco-Epidemiology** K. Kapo, Montani Run, LLC; S. Dyer, The Procter & Gamble Company, Central Product Safety, The Procter & Gamble Company, Miami Valley Innovation Center. Integration of existing data resources (e.g., local, state, and federal agencies, trade associations, etc.) for the identification and prioritization of ecosystem stressors over large geographic scales is a practical first-tier strategy for meeting the challenge of regional watershed management. Spatial applications of eco-epidemiology, in which geographic patterns of biological and diverse environmental parameters (e.g., percent effluent, habitat, river size, landcover, etc.) are related and expressed in quantitative terms of relative ecological risk, provide a desktop



approach for integrating and utilizing multiple agency data products and modeling resources. This presentation provides an overview of recent applications of a spatial analysis approach using weights-of-evidence and logistic regression, focusing on a statewide eco-epidemiological assessment of fish and invertebrate communities of Ohio. A 2000–2008 database of biological, habitat, chemical, toxicological, demographic, and other landscape factors from multiple agency resources linked to hydrologic features was analyzed to delineate stressor-response associations and ecological risk models at statewide and ecoregional geographic scales. The assessment of ecological risk from a holistic, large-scale perspective allowed for the evaluation of risk of specific chemicals within the realistic context of multiple ecosystem stressors, such as physical habitat stress. Variation in analysis design by predominant land use, stream size, and biological endpoints (species, traits, and community indices) provided a spatially explicit, comprehensive screening-level assessment of ecological risk that can serve as guidance for targeted assessment at the site-specific level. Such an analysis was found useful for determining experimental design requirements for investigating causal relationships of chemicals emanating from municipal wastewater treatment plants, such as those from consumer products.

**104 Integrated Multiple Stressor Regional Risk Assessment for the South River and Upper Shenandoah River, VA** W.G. Landis, Western Washington Univ, Institute of Environmental Toxicology, Western Washington Univ, Institute of Environmental Tox. & Chem.; K. Kolb Ayre, Western Washington Univ, Institute of Environmental Toxicology; A.J. Markiewicz, Western Washington Univ, Institute of Environmental Toxicology; J. Stinson, H.M. Summers, Western Washington Univ, Institute of Environmental Toxicology. An integrated risk assessment has been performed for the South River from the area just upstream of Waynesboro VA to the uppermost part of the Shenandoah River. Six risk regions have been delineated. The area is a site of historic mercury contamination from synthetic fiber production in Waynesboro. Other sources of other stressors include urban and agricultural run-off, channelization, erosion, and contaminated sediments and biota. The current iteration of the relative risk model (RRM) incorporating the hierarchical patch dynamics paradigm was used to construct a conceptual model, designate risk regions, create a ranking system and calculating risks to the stakeholder derived endpoints and impacts. In concert with this effort a Bayesian network was constructed based upon the framework of the RRM and using the same risk regions, sources, stressors, habitats and impacts. The use of the Bayesian network allows a straightforward calculation of management requirements to reduce risk in each of the six risk regions. Atypically, the current source of the Hg contamination is the environment itself. The warm water fish species have been found to have consistently high tissue concentrations downstream of the original source. Temperature may also be a risk factor affecting fish reproduction. Nutrients from upstream of Waynesboro also may be contributing to risk to a variety of endpoints. Although intensively studied for a number of years, the lack of data from upstream and from within the watershed are contributing to the uncertainties in the risk estimates. Much of the information regarding non-point sources has to be inferred from remote sensing estimates of the various types of land-cover for the risk regions. This research program identifies risks and demonstrates the benefits of an integrated risk, research and management program in the restoration of watersheds at a regional scale.

**105 Perfluorinated Compounds in Surface Waters from Northern China: Comparison to Level of Industrialization** T. Wang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China, State Key Lab of Urban and Regional Ecology; C. Chen, Chinese Academy of Science, Research Center for Eco-Environmental Sciences; J.E. Naile, Univ of Saskatchewan, Dept of Veterinary Biomedical Sciences and Toxicology Centre; J. Khim, Korea Univ; Y. Lu, Chinese Academy of Sciences; J.P. Giesy, Univ of Saskatchewan. Inclusion of Perfluorooctane Sulfonate (PFOS) into the Stockholm Convention has, because of its exemptions, has resulted in increased annual production of PFOS-containing chemicals in China to accommodate domestic and overseas demands. Accordingly, concern about environmental contamination with perfluorinated compounds (PFCs), such as PFOS, has been arisen. However, little information is available on the status and trends in the distribution, sources and risk of PFCs in aquatic environments of China. In the present study, forty two surface water samples collected from five regions with different levels of industrialization were monitored for concentrations of PFCs by use of solid phase extraction and LC/MS/MS. Mean concentrations

(maximum concentration) of PFOA and PFOS, which were the dominant PFCs, were 1.2 (2.3) and 0.16 (0.52) ng/l for Guanting, 1.2 (1.8) and 0.32 (1.1) ng/l for Hohhot, 2.7 (15) and 0.93 (5.7) ng/l for Shanxi, 6.8 (12) and 2.6 (11) ng/l for Tianjin, 27 (82) and 4.7 (31) ng/l for Liaoning, respectively. The greatest concentrations of PFCs (121 ng/l), PFOA (82 ng/l) and PFOS (31 ng/l) were observed in Liaoning, which might originate from tributaries of the Liaohe River, the most polluted watershed in Northeast China. While, concentrations of PFCs in the Guanting and Hohhot regions were 3 to 20 fold less than those from Tianjin and Liaoning. This result is consistent with little contribution of PFCs being released from agricultural and non-industrial activities. The magnitudes of mass flow for PFOA and PFOS in decreasing order were: Guanting < Hohhot < Tianjin < Liaoning < Shanxi and Guanting < Hohhot < Shanxi < Tianjin < Liaoning. The larger mass flows of PFOS were accompanied by relative larger magnitudes of PFOA. Concentrations of both PFOA and PFOS in waters from all regions were less than suggested allowable concentrations. However, the relatively greater concentrations of PFCs in Tianjin and Liaoning suggest that further studies characterizing their sources and potential risk to both humans and wildlife are needed.

**106 Application of Multiple Index Development Approaches to Benthic Invertebrate Data from the Virginian Biogeographic Province, USA** M. Pelletier, National Health and Environmental Effects Research Laboratory, USEPA, Dept: ORD NHEERL AED; A. Gold, Univ of Rhode Island, Natural Resource Science; L. Gonzalez, Univ of Rhode Island, Computer Science and Statistics; C. Oviatt, Univ of Rhode Island, Graduate School of Oceanography. Benthic invertebrate indices have commonly been utilized to assess benthic invertebrate communities. These indices have been constructed using different techniques, but have shown different levels of application success. For example, the EMAP Virginian Province Index did not perform well in a smaller estuarine complex. Similarly, the Chesapeake Bay Benthic Index of Biotic Integrity did not perform well outside of Chesapeake Bay, despite multiple metric and good habitat separation. In this study, we assembled multiple variables (metrics) from the literature and applied different methods of index compilation to explore the relative strengths and weaknesses of the indices. Three different approaches were utilized -- two multimetric indices (Chesapeake Bay approach and the Mebane approach) and a logistic regression technique. The data were subdivided by habitat (salinity and grain size) and indices compiled using the same initial group of benthic metrics. Each approach was examined for its classification accuracy for both reference and impaired sites for the entire Virginian Province. The Chesapeake Bay approach did not perform well in this study. In contrast, another multimetric approach, the Mebane approach, performed well, as did the logistic regression approach. Both techniques have promise for index development and could be useful in applying a biological condition gradient to estuaries.

**107 Integrated Assessment of Sediment Contamination by Anthropogenic Pollutants in a Semi-enclosed Bay in Korea** S. Hong, Korea Ocean Research & Development Institute, Oil and POPs research group; W. Shim, U. Yim, Korea Ocean Research and Development Institute, Oil and POPs research group; S. Ha, Korea Ocean Research and Development Institute; U. Yim, Korea Ocean Research and Development Institute, Oil and POPs research group; D. Lim, J. An, N. Kim, Korea Ocean Research and Development Institute. Marine environment is the ultimate sink for land based anthropogenic pollutants. After the adsorption to the suspended particles, the contaminants sink and stay remained at the bottom sediment for a long time. Benthic biota will eventually pickup these residues and biomagnify through the food web. In order to assess the contamination status and identify the priority substances in Masan Bay, intensive sediment sampling was conducted and organic and inorganic contaminants were analyzed. Additionally, benthic amphipod toxicity test was conducted as well. The spatial distribution of contaminants clearly revealed the fact that sewage treatment plant outfall and industrial waste output from the inner bay are major pollution sources. After the implementation of 'Total Pollution Loads Management System (TPLMS)' to this bay in 2008, the overall trend in sediment contamination decreased except for PBDE. In order to assess the pollution status of this bay in an integrative way, sediment quality index (SQI) was prepared from the chemical data. The SQI was calculated using two functions viz. 'scope' (the number of variables that do not meet guideline objective) and 'amplitude' (the magnitude by which variables exceed guideline objective) based on sediment quality guideline

(SQG) values from Canada, USA and Australia/New Zealand. The overall status of this bay is 'fair' or 'good'. Among the targeted chemicals, TBT and heavy metals were identified as the priority chemicals influencing the SQI values in Masan Bay. The SQI values were strongly influenced by the target variables (compounds) and their sediment quality guideline values. High copepod toxicity was also observed near the STP outfall and inner most part of the bay, which matched well with the spatial distribution pattern of toxic chemicals. However, some samples with high copepod toxicity didn't show high exceedance of SQG, implying co-contaminants, other than the target chemicals influence the motility.

#### **108 The Use of a Bayesian Network to Model the Risks of Mercury Contamination to Fish and Other Vertebrates in the South River, Virginia**

**H. Summers**, W. Landis, Western Washington Univ, Institute of Environmental Toxicology and Chemistry. The South River watershed in western Virginia has had a history of mercury contamination from past industrial practices. An environmental risk assessment of the effects of the mercury contamination was conducted using Bayesian networks. A conceptual model has been created for the effect pathways connecting the sources of mercury to the potential environmental impacts by examining the exposure through various habitats. From this conceptual model, a three-tiered Bayesian network was created for mercury impacts, which included sources of mercury contamination, habitats and direct/indirect toxicological effects on fish, amphibians, mammals and birds. Each parameter in this model consisted of four different risk levels: zero, low, medium and high. A method for evaluating data quality was created that characterizes their applicability to the stakeholder defined endpoints and potential uncertainties in order to categorize the potential for risk within each parameter. A series of distributions of data specific to the South River study area were used to create cumulative probability tables that were linked within the Bayesian network to calculate overall risk to stakeholder endpoints. The method was initially applied to a single endpoint, the risk to smallmouth bass (*Micropterus dolomieu*), for which the largest amount of sampling data exists. The model was expanded to other fish, amphibians and birds. A sensitivity analysis was conducted to identify the effect pathways with the greatest influence on smallmouth bass survival. The results of the analysis will inform future decisions for the restoration and management of the South River watershed.

#### **109 SETAC Gulf of Mexico Oil Spill Focused Topic Meeting: I. Summary on Ecosystem and Risk Assessment**

**W.L. Goodfellow**, EA Engineering Science & Technology, Inc.; **M.S. Greenberg**, USEPA, Environmental Response Team; **B. Vigon**, SETAC North America; **G. Schiefer**, SETAC North America. The SETAC Gulf of Mexico Oil Spill Focused Topic Meeting was held April 26–28, 2011 in Pensacola, FL, USA, attracting more than 250 participants. The meeting provided a multi-stakeholder forum for exchange of current and developing knowledge on key aspects of the spill and attracted a diverse group of oil spill assessors and responders with expertise in ecology, toxicology, chemistry, modeling and tracking of oil, technology development, emergency response, environmental management and risk communication. The assessment of the environmental impacts from the oil spill were presented in several sessions including the ecosystem effects of oil spills; risk and damage assessment; and seafood contamination, safety and human health issues. This first of two talks will summarize the discussions from these sessions related to the ecosystem assessment, risk and damage assessment and seafood safety and human health sessions. Included in this presentation will be a summary of the discussions on the assumption of the use of dispersants and the net environmental benefit analysis. These discussions indicated that the participants felt that a rigorous analysis of the risks and benefits of dispersant use in spill response is warranted, given the myriad of receptors and habitats, exposure regimes, and modes of effect. Developing better information to support decisions on the use of oil dispersants will require a holistic and integrated approach. Regarding seafood safety, the results presented indicated that seafood samples were 100–1000 times below levels of concern for PAHs and dispersant markers. A segment of one panel discussed the adequacy of the equivalency approach used, in that the number and relative toxicity of the individual PAH compounds taken into account results in a lower margin of safety than was claimed/presented in public forums (still below thresholds but less than the reported 100 to 1000 times). It was also asserted during the meeting that even though health agencies have determined based on extensive data that Gulf seafood is safe to eat, many people don't accept that. This presentation will highlight these and other key findings, data gaps and future research

needs identified within meeting sessions and panel discussions that focused on ecosystem impacts, ecological and human health risk associated with the Gulf of Mexico oil spill.

#### **110 SETAC Gulf of Mexico Oil Spill Focused Topic Meeting: II.**

**Summary on Current Response Technology and Capabilities** **M.S. Greenberg**, USEPA, Environmental Response Team; **W.L. Goodfellow**, EA Engineering Science & Technology, Inc.; **B. Vigon**, SETAC North America; **G. Schiefer**, SETAC North America. The SETAC Gulf of Mexico Oil Spill Focused Topic Meeting was held April 26–28, 2011 in Pensacola, FL, USA. The meeting provided a multi-stakeholder forum for exchange of current and developing knowledge on key aspects of the spill and attracted a diverse group of oil spill assessors and responders with expertise in toxicology, chemistry, modeling and tracking of oil, technology development, emergency response, environmental management and risk communication. Successful oil spill response relies on a multi-disciplinary approach and the application of numerous response techniques that may include subsea containment, remote sensing, mechanical recovery, surface and subsea use of dispersants, in situ burning, and natural attenuation. These techniques are each most efficient under a different conditions (e.g., oil properties, release scenarios, environmental conditions) and should all be evaluated for their applicability in dynamic spill response situations. An understanding of oil fate and transport is a key input factor needed for this evaluation. Current knowledge on oil transport indicated that this occurred in the Gulf within two main regimes: a subsea plume of dispersed particles (< 100  $\mu$ m) and associated dissolved compounds; and a surface plume (>1 mm). The fate of the oil within these regimes (e.g., degree of weathering, bioavailability, oiling of shorelines and the sea bottom) was different as evidenced by the monitoring, chemistry, and environmental forensics data that were presented during the meeting. Several panel discussions centered on improving or increasing the effectiveness of the response toolbox, and how to better choose among response actions. Other discussions identified a combination of needs from higher resolution data on particle size distributions to a variety of fate and effects modeling issues. Some modeling conversations focused on environmental compartments that are not well understood, while others discussed overall improvements to the multi-media integrated models themselves. There was consensus that a better understanding of oil fate will also improve our ability to predict ecosystem-level effects of the spill. This presentation will highlight these and other key findings, data gaps and future research needs identified within meeting sessions and panel discussions that focused on control and abatement techniques, oil fate and transport measurement and modeling, oil tracking technology, and response technology effectiveness.

#### **114 Species Composition, Distribution and Abundance of Megafauna in the Vicinity of MC252 During August 2010 and March 2011**

**M. Benfield**, Louisiana State Univ, Oceanography and Coastal Sciences; **R. Putt**, Environmental Consultant; **M. Valentine**, Louisiana State Univ. This presentation will provide data from two independent surveys conducted in close proximity to the Macondo well BOP. From Aug–Sep 2010, two ROVs were used to survey the distribution and abundance of megafauna at 5 sites around the MC252 well. Five sites were located 2000 m due N, W, S, E of the well and 500 m N of the well. Two of these sites (2000 m N, W) were resurveyed during March 2011. A series of radial 250m transects on bearings separated by 15° (Aug 2010) or 30° (Mar 2011) were conducted. Densities of benthic and demersal megafauna were quantified at each site. The sites were dominated by seastars, sea pens, crustaceans (shrimp, crabs, and squat lobsters), and a variety of fish species. Comparisons with independent, qualitative, pre-spill ROV surveys conducted during Feb/Mar 2010 at MC252 suggest similar species dominated before and after the spill. Statistical comparisons among sites will be summarized to compare mean densities for dominant taxa.

#### **116 Modifications to Diets Greatly Affect Outcomes in the Amphibian Metamorphosis and the Fish Short Term Reproduction Assays**

**H. Krueger**, Wildlife International, LTD, Aquatic Toxicology; **J. Claude**, Wildlife International, LTD, Aquatic Toxicology; **T. Ross**, S. Gallagher, Wildlife International, LTD; **S. Schneider**, S. Palmer, E.M. Burgess, Wildlife International, LTD, Aquatic Toxicology; **T. Springer**, Wildlife International Ltd; **A. Leopold**, Leopold Ecotox Services, Wildlife International Ltd.; **M. Jaber**, Wildlife International, LTD. Slight modifications to the diets specified in the new test guidelines for both the Fish Short Term Reproduction and

Amphibian Metamorphosis Assays improved our ability to meet the performance and validity criteria of the tests. In our laboratory, we found that strict adherence to the feeding regimes of both guidelines was too restrictive. For the fish assay, we could not consistently meet the fecundity requirements using the feeding regime from the guideline. Feeding trials were performed comparing the fish guideline diet of frozen adult brine shrimp to a modified diet that combined newly hatched brine shrimp with commercial flake food. Fish were better able to sustain reproductive output under the modified diet, which has allowed us to more consistently meet guideline acceptance criteria. Feeding trials with tadpoles compared the guideline rates of Sera Micron diet to ½ those amounts. Use of the guideline rates for amphibians resulted in accelerated growth and development with more frogs being pushed into later stages of development (greater than NF Stage 60) at test termination. A high incidence of tail curvature or scoliosis in tadpoles also was observed at the guideline rate. By reducing the feeding rate to approximately ½ the guideline rate, the amount of tail curvature or scoliosis was greatly reduced and tadpoles were at developmental stages that better fit the acceptance criteria of the guideline.

**117 Summation of Historical Control Data for Both the Amphibian Metamorphosis Assay and the Fish Short Term Reproduction Assay** K. Coady, The Dow Chemical Company, Toxicology & Environmental Research and Consulting, Michigan State Univ, Zoology Dept; R.J. Currie, The Dow Chemical Company, Toxicology, Environmental Research and Consulting; T. Marino, C. Lehman, The Dow Chemical Company; V.J. Kramer, Dow AgroSciences, LLC; J. Thomas, L. McFadden, The Dow Chemical Company; G.M. Klecka, The Dow Chemical Company, Toxicology and Environmental Research Dept. The amphibian metamorphosis assay (AMA) and the fish short term reproduction assay (FSTRA) are both Tier 1 tests in USEPA's Endocrine Disruptor Screening Program. In the Tier 1 battery of tests, the AMA is intended to assess the potential activity of test chemicals on the hypothalamus-pituitary-thyroid (HPT) axis of developing African clawed frogs (*Xenopus laevis*), while the FSTRA is intended to assess both (anti-)estrogenic and (anti-)androgenic activity as well as potential activity in the hypothalamus-pituitary-gonad (HPG) axis of fathead minnows (*Pimephales promelas*). Our lab has run eleven AMA and eleven FSTRA tests over the last several years, and historical control data from these tests are summarized and compared to the USEPA and the Organisation for Economic Co-operation and Development validation data sets. Variability in the measured endpoints is assessed and the ramifications for the study designs, test performance criteria, and interpretations of findings are considered for each assay.

**118 EDSP Implementation: Impact of OSRI Acceptance and Path Forward for Ecotoxicology Assays** W. Jones, M. Leggett, CropLife America; M. Kern, Bayer CropScience; C.J. Borgert, Applied Pharmacology and Toxicology. In response to congressional mandates in 1996, EPA developed the endocrine disruptor screening program (EDSP) with the intent to identify chemicals with the potential to interact with the estrogen, androgen, or thyroid systems. EDSP is a tiered approach with Tier I providing the screening battery and Tier II providing more definitive multi-generational studies. In late 2009, EDSP was officially implemented with the receipt of testing orders for the chemicals on the first screening list, the majority being pesticide active ingredients. Chemicals selected for screening are subjected to a battery of 11 assays. Test order recipients could choose to utilize existing information- also called Other Scientifically Relevant Information (OSRI) – in lieu of generating new data for a sub-set or the entire Tier I battery. Of the 50+ pesticide registrants, 61% provided OSRI for the in vivo EDSP Tier I studies. Of those that cited OSRI for the Amphibian Metamorphosis assay and the Fish Short Term Reproduction Assay, the USEPA rationale for acceptance and rejection of the OSRI will be discussed; only 4% of the Fish Short Term Reproduction Assay waivers and 13% of the Amphibian Metamorphosis Assay waivers were accepted. Understanding the agency's rationale for accepting or rejecting data submitted as OSRI will be increasingly important as this rationale directly affects animal use and the overall resource burden of the EDSP program. Of emphasis will be how consistently the OSRI was evaluated, what were the missing endpoints within the OSRI submitted and to what extent would other assays in the Tier I battery address those endpoints (or other Part 158 studies). The discussion will include challenges associated with the Amphibian Metamorphosis Assay and the Fish Short Term Reproduction Assay according to the prescribed Series 890 Test Guidelines (including performance criteria). With the focus of

this first phase of EDSP on data-rich pesticides, learning's from this listing of chemicals can help industry, USEPA and other stakeholders on the path forward.

**119 Comparative Sensitivity of Fish to EDSP Testing and Standard USEPA Pesticide Testing** C. Habig, Exponent, Inc., Compliance Services, Inc., Principal Scientist; P. Whatling, Cheminova, Inc., Senior Manager of Regulatory Science. EPA has recently initiated endocrine disruptor screening testing on chemicals to determine whether the test chemicals display effects on selected endocrine systems. A key tenant of the new requirement for endocrine testing is that organisms are highly sensitive to endocrine-active substances, and thus, effects due to these types of substances may be missed in USEPA's standard pesticide guideline testing. Tier I endocrine testing consists of a battery of in vitro and in vivo assays designed to determine whether the test compounds interact with either the reproductive system or thyroid-mediated processes. These assays include two assays involving non-target organisms, the fish short-term reproduction test and the amphibian metamorphosis test. USEPA's initial list of compounds required to undergo endocrine disruptor (EDSP) testing primarily consisted of pesticides. Relatively full data sets were already available for the majority of these pesticides, including mammalian and avian reproduction testing, fish early life stage testing, daphnid and mysid lifecycle testing, and, in some cases, fish full lifecycle testing and/or field testing (mesocosms). While some of the EDSP test endpoints differ from those evaluated in these standard guideline tests, some of the endpoints are either the same, or essentially similar. This presentation reviews the results of EDSP testing on an OP insecticide using fathead compared to previous results from a fish early life stage study with rainbow trout, and results from preliminary fathead minnow full lifecycle testing. The current chronic toxicity risk assessment endpoint for this compound is based on the results of a trout early life stage (ELS) test. The preliminary fathead minnow full life stage testing indicated that fathead minnows are substantially less sensitive to this OP than trout. Results of the trout ELS indicated that the most sensitive endpoint was fish growth (length and weight). Results of the EDSP testing confirmed that fathead minnows are less sensitive than trout to this OP. Moreover, the fathead EDSP results indicated that length and weight were still the most sensitive endpoints, but at concentrations substantially higher than in trout. There were no effects on the reproductive endpoints assayed in the EDSP study at test concentrations that did not elicit observable toxicity in the test organisms.

**120 Results of Tier 1 Screening of the USEPA Endocrine Disruptor Screening Program Compared to Data Generated for Pesticide Registration** A. Blacker, C. Banman, M. Dobbs, Bayer CropScience; L.S. Ortego, Bayer CropScience, Ecotoxicology; L. Sheets, Bayer CropScience. Pesticides are among the first compounds undergoing Tier 1 testing in USEPA's Endocrine Disruptor Screening Program (EDSP). The Tier 1 testing battery consists of 11 assays designed to identify whether a chemical has the potential to interact with the estrogen, androgen or thyroid pathways of the endocrine system. Under the Federal Insecticide, Fungicide and Rodenticide Act and subsequent amendments, comprehensive toxicology and ecotoxicology testing is required for registration of a pesticide. The data generated in the registration process includes sub-chronic and chronic testing evaluating growth, development and reproduction in multiple species and provides valuable information on whether a compound has potential endocrine activity. However, Tier 1 assays do include endpoints, e.g., receptor binding, vitellogenin, which are not measured in the current data requirements for a pesticide registration. Comparisons of Tier 1 results to toxicity data for registration purposes will be provided using specific examples, and utility of the 11 Tier 1 screens will be discussed in relation to existing datasets for pesticides.

**121 Application of an Integrated Testing Strategy to the USEPA Endocrine Disruptor Screening Program Could Reduce Aquatic and Other Animal Testing** C. Willett, P. Bishop, People for the Ethical Treatment of Animals, Regulatory Testing; K. Sullivan, Physicians Committee for Responsible Medicine. To maximize efficiency and reduce the number of animals used in testing, new approaches to generating and evaluating toxicity data for chemicals are needed to cope with the ever-increasing demands of new programs like the US Environmental Protection Agency's (USEPA) Endocrine Disruptor Screening Program (EDSP). One such approach involves the use of an integrated testing and evaluation strategy based on the specific properties and activities of a chemical. In this approach, testing



is organized according to the type of information generated. At each step, the information is then reviewed and used to target any potential further testing, until the information necessary for hazard or risk assessment has been generated. Such an integrated strategy, whether applied to existing or future programs, can promote efficient use of resources and save animals. We compare the numbers of tests that will be performed and animals used in the first phase of the current EDSP with what the outcome could have been if the proposed strategy had been used for three randomly selected chemicals.

**122 Cross-Species Extrapolation of EDC Toxicity: Consequences for Screening Programs** G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; L.E. Gray, USEPA. Many structural and functional aspects of the vertebrate hypothalamic-pituitary-gonadal (HPG) axis are known to be highly conserved, but the full significance of this from a toxicological perspective has received comparatively little attention. High-quality data generated through development and validation of different Tier 1 tests for the USEPA Endocrine Disruptor Screening Program (EDSP) offer a unique opportunity to compare responses of mammals versus fish to chemicals that affect shared pathways within the HPG axis. This analysis used data from 12 different chemicals that act (primarily) as estrogen receptor agonists (17 $\alpha$ -ethynylestradiol, methoxychlor, Bisphenol A), androgen receptor agonists (methyltestosterone, 17 $\beta$ -trenbolone), androgen receptor antagonists (flutamide, vinclozolin, *p,p'*-DDE) or inhibitors of different steroidogenic enzymes (ketoconazole, fadrozole, fenarimol, prochloraz) that had been tested in the 21-d fathead minnow assay and in one or more of the other four *in vivo* (rat) Tier 1 screens (Uterotrophic, Hershberger, male and female pubertal assays). Each of the 12 chemicals was identified as endocrine-active by two or more of the five Tier 1 assays, indicating excellent coverage of the HPG pathways of concern. The fathead minnow assay was positive for all the test chemicals. The consequences of these observations in terms of screening efforts like the USEPA EDSP will be discussed. The contents of this abstract do not reflect USEPA policy.

**123 The Development of Quantitative Weighting Factors for Use in a Weight of Evidence Framework for the Evaluation of Endocrine Activity** C. Borgert, Applied Pharmacology & Toxicology, Inc.; E. Mihaich, Environmental and Regulatory Resources, LLC; L. Ortego, Bayer CropScience; K. Bentley, DuPont Crop Protection; C. Holmes, BASF Corporation; S. Levine, Monsanto Company; S. Marty, Dow Chemical Company; B. Neal, Exponent; R. Becker, American Chemistry Council. We previously developed a conceptual framework for conducting weight of evidence (WoE) evaluations of endocrine screening and testing data (Borgert et al., *in press*). The framework was designed to be broadly applicable, but with an emphasis on data emerging from the USEPA's Endocrine Disruptor Screening Program (EDSP), and specifically tailored to accommodate endpoints measured in the eleven Tier 1 Endocrine Screening Battery (ESB) assays. The framework calls for a transparent process whereby specific hypotheses are formulated and data quality is evaluated with respect to overarching scientific principles, including primary validity of the measurements, reliability of data reporting, and probative capability of the study design to evaluate causation. The framework also calls for assigning quantitative weighting factors to assay endpoints (WREL) that reflect their relevance for deciding specific hypotheses. This is an important step in the WoE framework as it explicitly recognizes the fact that some assays are very specific and sensitive for evaluating certain hypotheses related to endocrine activity but may be irrelevant for evaluating others. For example, an increase in thyroid follicular hyperplasia as assessed in the amphibian metamorphosis assay would be expected to have a very high WREL for evaluating the hypothesis that a chemical has potential thyroid antagonist activity, but would have a very low WREL for evaluating the hypothesis that a substance has potential estrogen or androgen agonist or antagonist activity. The framework recommends that WREL values be derived as numerical weighting factors based on the predictive value of the endpoint for actual endocrine activity *in vivo*, which would typically be determined in chronic reproductive and developmental toxicity tests, i.e., EDSP Tier 2 tests. This predictive value, however, may be difficult to evaluate until results for a test set of chemicals are available from the ESB and can be compared to results of Tier 2 tests. As an interim step, however, provisional WREL values can be derived based on a rank ordering of relevance for each hypothesis. This rank ordering should also be based on data, but will necessarily involve considerable scientific judgment. An example derivation of rank ordered WREL values for evaluating Tier 1 EDSP

hypotheses is presented, with particular emphasis on endpoints measured in the fish screening and frog metamorphosis assays.

**124 A Comparison of Fish BCFs Obtained for Pharmaceuticals as per OECD 305 and Estimated BCFs Obtained Using the Reduced Sampling Method** L.A. Constantine, Pfizer, Inc., Pharmacokinetics, Dynamics and Metabolism. Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. Such studies are typically considered appropriate for pharmaceuticals having a logD value > 3 and required, as per the EMA Guideline, for pharmaceuticals having logD values > 4.5. The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to 1/2 the duration of the uptake phase. During the uptake phase, ranging between 14 and 60 days, water and fish samples are analyzed periodically until steady state has been established and the depuration phase may be initiated. During the depuration phase, analysis of water and fish samples continues until a plateau has been reached. Based on the sampling schedule outlined in the guideline, >100 fish per study may be required to determine a kinetic bioconcentration factor (BCF). In an effort to reduce the number of fish required to obtain a BCF, an approach to estimating the aquatic bioconcentration factor using reduced sampling has been developed (Springer, et al. 2008). This reduced sampling approach aims to estimate BCFs using a minimum number of sampling time points and therefore a reduced number of fish. The goal of this project is compare the BCFs of several active pharmaceuticals obtained through standard OECD 305 test methods with those BCFs determined using the approach of estimating aquatic bioconcentration factors using the reduced sampling method.

**125 Derivation of Screening Values for Ecological Risk Assessment of PPCP Releases from CSOs into the Gowanus Canal, Brooklyn, NY** S.D. Baker, GEI Consultants, Inc., Ecotoxicologist/Wildlife Biologist; N.E. Paden, GEI Consultants, Inc., Ecological Division; N. Love, C. Claytor, S.M. Pargee, GEI Consultants, Inc.; R.W. Gensemer, GEI Consultants, Inc., Ecological Division. Screening values were derived for pharmaceutical and personal care products (PPCPs) as part of a Screening Level Ecological Risk Assessment (SLERA) to evaluate the potential for ecological risks in the Gowanus Canal due to loadings from combined sewer overflow (CSO) discharges. For each media sampled (i.e., water and sediment), conservative screening values for PPCPs detected at least once were derived. When available, previously-generated, risk-based thresholds from national or international government documents (e.g., US Environmental Protection Agency [EPA] and Canadian Council of Ministers of the Environment) which have undergone peer- and public-review were used; however, very few such efforts have been conducted for PPCPs. For PPCPs without existing thresholds, the primary literature was searched for relevant toxicity data. The approach used to derive screening values depended on the amount of relevant toxicity data available for a given PPCP. Several PPCPs had limited available toxicity data, but in a few cases, many data for several species were available. If data were not available from the literature, thresholds of toxicity as predicted by EPA's ECOSAR model (EPA 2003) were used as screening values. In general, sediment toxicity data were less available than those for surface water, so sediment screening values could be derived from available toxicity data for only a few chemicals. Therefore, sediment screening values were generally estimated from surface water screening values using organic carbon partition coefficients (K<sub>oc</sub>) and the fraction of organic carbon (f<sub>oc</sub>) to predict equilibrium partitioning between sediment and overlying water. The detailed processes and decision-making criteria used to derive surface water and sediment screening values for almost 30 PPCPs will be presented.

**126 Environmental Impact Analysis of 10 Active Pharmaceutical Ingredients with QSARs** L. Geerts, Flemish Institute for Technological Research NV ("VITO"); I. Van Ginneken, B. Mertens, Janssen Pharmaceutical Companies of Johnson & Johnson; D.J. Caldwell, Johnson & Johnson. An environmental risk assessment is required for all new marketing authorisation applications of medicinal products. The risk assessment follows a stepwise approach. As a start a base set on aquatic toxicology and fate of the active pharmaceutical ingredient (API) has to be generated. This base set can be generated by tests performed according to the standard OECD guidelines, but before doing so the potential effects on aquatic organisms and the environmental fate can be calculated to screen for possible alerts. Structure activity relationships (SAR) are methods for estimating properties

which provide information on intrinsic hazards of chemicals, while reducing time, monetary cost and animal testing. Ten APIs from multiple therapeutic categories were evaluated using EpiSuite. Model results were compared to measured values. QSARs are shown to be a reliable tool for screening environmental properties of APIs. All the test result on (non) ready biodegradability were confirmed by the predictions of the Biowin model. All the ecotoxic substances were identified by Ecosar. The acute toxicity is in line with the measured values (74%) or overestimated (26%) by the model. For the chronic toxicity, the lowest chronic value determines the PNEC and hence the outcome of the risk characterisation. The calculated lowest chronic value for each API is in line with the measured value or lower. For the Ecosar classes considered for these 10 APIs, the main parameter in the algorithm is the log K<sub>ow</sub>: the higher the log K<sub>ow</sub>, the lower the ecotoxicity value. It could be argued for the salts to provisionally use the measured log K<sub>ow</sub> in order to refine the overestimation and avoid underestimation of the ecotoxicity by the model. For the APIs with only covalent bonds the results with measured and calculated ecotoxicity are in the same order of magnitude. As part of an intelligent testing strategy within risk assessment, QSAR predictions can deliver added value to other non-test methods such as in vitro tests, read across and a weight of evidence approach in order to replace animal testing where justified and to secure a high level of safety for man and the environment.

**127 Pharmaceuticals at Sites Affected by Contamination: A UK Approach to Assessing Risk to Human Health** K. Baker, J. White, B. Magee, ARCADIS. The UK has a well developed framework for the assessment of risks to human health and the environment from land contamination. This includes good practice guidance on deriving Health Criteria Values (HCV), or acceptable daily intakes, for use in quantitative risk assessment and to generate clean-up criteria. However, limited guidance is provided within the framework to assist in developing HCVs where suitable toxicological studies are not readily available. For facilities involved in the production of pharmaceuticals, the absence of data required to derive HCVs in line with the good practice guidance has resulted in poor, or even no, assessment of the potential risks from these compounds where present in soil or ground-water following leaks or spills. A case study for a site in the UK is presented, which overcame these difficulties by calculating HCV utilizing Minimum Therapeutic Doses, an approach initially proposed by the UK Drinking Water Inspectorate. The use of the methodology enabled assessment of risk to human health from 11 pharmaceutical compounds, including phenobarbital, chlorpromazine and molindone, and the derivation of clean-up goals to assist in the remediation process.

**129 Environmental Risk Assessment of Ionisable Organics** T. Gouin, Unilever, Safety and Environmental Assurance Centre; V. Coombe, Astra Zeneca; J.F. Ericson, Pfizer Inc, Environmental Sciences, PDM; B. Hidding, BASF; J. Ryan, GlaxoSmithKline; K. Silverman, Merck & Co., Inc., Global Safety & the Environment; B. Simon-Hettich, Merck KGaA. The environmental release of ionisable organic compounds (where behaviour depends on pH, ionic strength, etc.) represents a number of challenges for risk assessors who must select relevant and appropriate test endpoints using methods that have historically been used with more neutral lipophilic compounds. ECETOC have thus established a Task Force to review the current understanding and available literature on partitioning property data of ionisable organic compounds at environmentally relevant pHs, including estimation methods for these properties. It is anticipated that the output of the task force will lead to suggestions and recommendations for improved methods for predicting the environmental concentration of ionisable organic compounds in aquatic environments. To date, the work of the Task Force has emphasised the need for better generic environmental fate models, with appropriate soil and water properties, and other parameters required for effective environmental risk assessment of ionisable compounds. Preliminary findings include identification of the key parameters needed to better predict the bioavailability of ionisable organic compounds, which are shown to be strongly influenced by the physical-chemical properties of the substance, for instance,  $pK_a$  and log  $K_{ow}$ , as well environmental properties such as pH. Modelling activity is supported from data assembled for a physical-chemical property database for ionisable organic compounds used as pharmaceuticals. Using the best available property data a sensitivity analysis has been performed using a number of environmental fate models to determine how variability in environmental input parameters influence the predicted environmental concentration and ultimately the bioavailability of a substance.

Recommendations on where future research priorities should be targeted are summarized.

**130 Magnifying Perceived Risk: Trace Concentrations of Pharmaceuticals in Surface and Drinking Water Have No Demonstrated Human Health Impact** D.J. Caldwell, Johnson & Johnson; F. Mastrocco, Pfizer, Inc.; P. Anderson, ARCADIS. Media reports have indicated the presence of pharmaceuticals in surface and drinking water with no details given on actual concentration. For some pharmaceuticals, such as steroid hormones, inaccurate field measurements reported in scientific journals have led to reports of "high concentrations" that are called 'environmentally-relevant' when they are not. Speculation about potential adverse effects from such exposures has produced undue concern and the potential risk from surface and drinking water exposure to pharmaceuticals is exaggerated. With the media frequently focusing on contraceptives use when discussing the endocrine issue, public perception is being swayed and the regulatory community is considering unwarranted risk management actions to address the presence of trace concentrations of pharmaceuticals with potentially costly consequences. While not as extensively evaluated as the steroid hormones, reports of detection of other pharmaceuticals in surface and drinking water and the selective reporting of these studies have contributed to the misconception that pharmaceutical exposure is of great consequence to humans. A detailed review of monitoring and effects data demonstrates human exposures to pharmaceuticals in surface and drinking water are minor and well below available acceptable daily intake values. We conclude that there is negligible impact on human health from exposure to trace concentrations of pharmaceuticals in surface and drinking water.

**131 Unique Challenges in the Health Risk Assessment of Antibiotic Resistance Genes in the Environment** A. Pruden, Virginia Tech, Via Dept of Civil and Environmental Engineering. Recent research has clearly revealed that human activities have a profound impact on the levels of antibiotic resistance in the environment. Wastewater treatment plants and animal feeding operations have been identified as two major contributors of elevated antibiotic resistance genes observed in impacted water bodies. However, the risks to human health imposed by environmental sources of antibiotic resistance remain elusive. Clearly, the rates of resistance among critical pathogens have been increasing at a global level, and antibiotic resistance remains as one of the most critical human health threats of our time. Unfortunately, current risk models are not adequate to assess the human health risks posed by antibiotic resistance genes. Typical risk assessments are based on fundamental dose-response relationships, which are not directly applicable to antibiotic resistance genes. Antibiotic resistance genes can persist independent of a host and transform new bacterial hosts. They may also establish among the natural gastrointestinal flora of humans and livestock, thus resulting in a latent risk of onset of an antibiotic resistant infection. Furthermore, within environmental reservoirs, the elevated background of resistance elements increases the probability that pathogens will acquire resistance and contribute to an overall higher background resistant pathogen exposure rate. Some exposure routes to consider include: ingestion of drinking water and dermal and respiratory contact during bathing or recreational water activities. The purpose of this presentation will thus be to outline the critical steps needed to move forward in the development of a risk assessment for antibiotic resistance genes in the environment.

**132 Cytochrome P450 CYP2- and CYP17-like Genes in the Mangrove Oyster *Crassostrea brasiliana*: Insights on Mechanism of Phenanthrene Toxicity in Bivalves** K.H. Luchmann, T. Dorrington, UFSC, Bioquímica; A.C. Baimy, Universidade Federal de Santa Catarina, Bioquímica. Polycyclic aromatic hydrocarbons (PAHs) have been recognized as typical inducers of cytochrome P450 (CYP) superfamily in vertebrates, which has become a common biomarker of PAH exposure. A limited number of studies are available on CYP modulation in bivalve. We show the presence of three new putative CYP genes from the mangrove oyster *Crassostrea brasiliana* and changes in their expression following short-term exposure to phenanthrene. CYP homologues were searched against the *C. brasiliana* EST database and sequences with identity >40% were annotated using blastx algorithm. Sequences corresponding to CYP2, CYP3 and CYP17 families were identified and transcript levels were evaluated by qPCR in the gill and digestive gland of oysters exposed for 24 h to 100µg and 1000µg of phenanthrene. Phenanthrene (1000µg) induced expression of CYP2-like gene in both tissues, while CYP17-like was induced in the gill. The largest change in response



to 1000µg phenanthrene were the induction of *CYP2-like* (~11-fold) and *CYP17-like* (~9-fold) in gill. Although also induced in digestive gland, *CYP2-like* mRNA levels were much less responsive (~2-fold induction) to 1000µg phenanthrene. In contrast to the other genes, *CYP3-like* was not induced by phenanthrene in any of the tissues or concentration. The tissue-specific response of *CYP17-like* and the higher transcripts of *CYP2-like* in gill suggest an important function of this tissue in PAH biotransformation for bivalve mollusks. These results represent the first insight into *CYP* transcript responses of *C. brasiliensis* with potential applicability in field surveys. Further studies are necessary to address roles for *CYPs* providing a better understanding of the biotransformation process in bivalve mollusks. Support: CNPq INCT-TA, FAPESP

**133 DNA Damage from Aflatoxin B1 but not Benzo[a]pyrene in *Caenorhabditis elegans*: A Case Study of Evolution of Genotoxicity** M.C. Leung, Duke Univ, Nicholas School of the Environment, Duke Univ, graduate student; J.V. Goldstone, Woods Hole Oceanographic Institution; W.A. Boyd, National Toxicology Program, Biomolecular Screening Branch; J.H. Freedman, National Institute of Environmental Health Sciences, Laboratory of Toxicology and Pharmacology; J.N. Meyer, Duke Univ, Nicholas School of the Environment. The nematode *Caenorhabditis elegans* is emerging as a useful model in toxicology, but there is little information on how it responds to different promutagenic compounds. We carried out experiments to elucidate the capacity of *C. elegans* to metabolically activate two important human promutagens via CYPs – aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), a naturally occurring mycotoxin found in foods such as corn, peanuts, various other nuts, and cottonseed and metabolized in mammals by CYP1, CYP2, and CYP3 family enzymes; and benzo[a]pyrene (BaP), an environmental pollutant produced by incomplete fossil fuel combustion and metabolized in mammals by CYP1 family enzymes. Phylogenetic comparisons identified CYP2 and CYP3 family enzymes in *C. elegans*, but not CYP1 family enzymes. While exposure to AFB<sub>1</sub> resulted in significant DNA damage in *C. elegans*, exposure to BaP produced no detectable damage. To further test whether BaP exposure generates bulky DNA adducts, the toxicities of AFB<sub>1</sub> and BaP were compared in nucleotide excision repair (NER)-deficient (xpa-1) and NER-proficient (N2) strains of *C. elegans*. Exposure to AFB<sub>1</sub> inhibited growth more in xpa-1 than N2 nematodes, but the growth-inhibitory effects of BaP were indistinguishable in the two strains. In addition, a CYP-nicotinamide adenine dinucleotide phosphate reductase-deficient strain (emb-8) of *C. elegans* was found to be more resistant to the growth-inhibitory effect of AFB<sub>1</sub> exposure than N2, showing that the AFB<sub>1</sub>-mediated growth inhibition resulted from CYP-mediated metabolism. Together, these results indicate that *C. elegans* lacks biologically significant CYP1 family-mediated enzymatic metabolism of xenobiotics. Finally, AFB<sub>1</sub> was found to selectively target mitochondrial DNA in *C. elegans*, which suggests a conserved mechanism of intracellular partitioning in nematode and mammalian species. The results also suggest a broader cross-species genotoxicity of the naturally-occurring AFB<sub>1</sub> as compared to the man-made BaP, possibly due to the selective pressure on the detritivore, AFB<sub>1</sub>-producing mold species.

**134 Identification and Expression of Genes in Response to Anthropogenic Stressors from the Estuarine Sea Anemone *Nematostella vectensis*** A.M. Reitzel, Woods Hole Oceanographic Institution, Biology; J.V. Goldstone, Woods Hole Oceanographic Institution; M.J. Jenny, Univ of Alabama; A.M. Tarrant, Woods Hole Oceanographic Institution. Estuaries are heavily impacted by a broad range of anthropogenic contaminants including metals, aromatic hydrocarbons, pesticides, and pharmaceuticals. The fate of estuarine communities depends upon the ability of resident organisms to deploy molecular and physiological responses to a combination of anthropogenic and natural stressors. Previous research has provided a wealth of data describing these mechanisms in fish and crustaceans, but we currently lack sufficient data on what mechanisms a majority of resident organisms, particular infaunal species, deploy to combat environmental stressors. We conducted bioinformatic surveys to identify the suite of genes from the estuarine sea anemone *Nematostella vectensis* that are potentially involved in stress responses (“defensome”). Broadly, these studies support a hypothesis that many genes are ancient and were present in the Cnidarian-Bilaterian ancestor. Secondly, we studied the relative toxicity of diverse chemicals to *Nematostella*. Thirdly, we employed molecular techniques, including transcriptional profiling and quantitative PCR, to determine the effects of toxic metals and hydrocarbon pollutants on gene expression for further insight into gene function. We have identified several genes that

may be useful bioindicators of environmental stressors. Together, these data provide an integrated view of the molecular mechanisms deployed by an infaunal invertebrate and provide an informative reference for studies of related species of conservation concern, particularly hard corals.

**135 Mitochondrial Fitness, Gene Expression, and Hypoxic Stress in a Hybrid Population of the Killifish, *Fundulus heteroclitus*** P.A. Flight, Brown Univ, Ecology and Evolutionary Biology; D. Nacci, USEPA, ORD, NHEERL, Atlantic Ecology Division; D. Champlin, USEPA; A. Whitehead, Louisiana State Univ, Dept of Biological Sciences; D.M. Rand, Brown Univ, Ecology and Evolutionary Biology. The physiological link between oxygen availability and mitochondrial function is well established. However, whether or not fitness variation is associated with mitochondrial genotypes in the field remains a contested topic in evolutionary biology. In this study we draw on a population of the teleost fish, *Fundulus heteroclitus*, where functionally distinct subspecies hybridize, likely as a result of past glacial events. We had three specific aims: 1) to infer the age of the split between the subspecies using mtDNA sequences; 2) to determine the effect of mtDNA genotype on survivorship of male and female fish under hypoxic stress; 3) to determine the effect of hypoxic stress, sex and mtDNA genotype on gene expression. Our results are consistent with previous studies suggesting the split between *F. heteroclitus* is more than an order of magnitude older than the last glacial maximum (~700 kya). We found a highly significant effect of sex on survivorship under hypoxic conditions ( $P=0.0022$ ), but no significant effect of mtDNA genotype. Gene expression analyses revealed hundreds of transcripts differentially regulated by sex and hypoxia. Mitochondrial transcripts were among those influenced by hypoxic stress, and the mtDNA control region was the most highly suppressed transcript under conditions of hypoxia ( $P=9.40 \times 10^{-8}$ ). Effects of mtDNA sequence variation on expression were limited, however a potentially important epistasis between mtDNA and a nuclear-encoded mitochondrial translation protein was discovered. Overall, these results confirm that mitochondrial regulation is a major component of hypoxia tolerance and further suggest that purifying selection has been the predominant selective force on mitochondrial genomes in these two subspecies.

**136 Molecular and Biochemical Response in the UV-B Damaged Copepod, *Tigriopus japonicus*** J. Lee, Hanyang Univ Graduate School, Dept of Chemistry; J. Rhee, Hanyang Univ, Dept of Molecular and Environmental Bioscience; Y. Lee, Sangmyung Univ, Dept of Green Life Science. The copepod mitochondrial genome showed a high divergence between biogeographically-isolated conspecific strains, indicating that there might be low DNA repair ability, resulting in accumulating mutation on the genome. To examine DNA repair ability and damage response, we measured photoreaction and dark repair ability after UV-B irradiation, and found that the intertidal copepod, *Tigriopus japonicus* has significantly poor activity for DNA repairs. To examine the ability of molecular chaperoning protein, heat shock proteins upon DNA damage, we performed acute toxicity test for UV-B radiation and measured ROS level and antioxidant enzyme (GR, GPx, SOD, GSH, and GST) activity after exposure to UV-B (12 and 24 kJ m<sup>-2</sup>) for 48 h in the copepod *T. japonicus*. Subsequently, transcriptional changes in hsp families (hsp10, 20, 20.7, 40, 60, 70, 70p, 90α, and 105) were further investigated in stress-exposed groups. As results, the LD50 value for UV-B radiation was measured at 23.12 and 26.42 kJ m<sup>-2</sup> in the male and female copepod for 48 h. ROS level was significantly increased at UV-B-exposed copepod. Activities of antioxidant enzymes were also highly elevated at both stressors-exposed copepods, indicating that UV-B can induce oxidative stress by generating ROS and stimulate the involvement of antioxidant enzymes as defense mechanisms against their toxicity. In experiment with UV-B radiation, the expression of hsp20 mRNA was firstly appeared at 6 kJ m<sup>-2</sup> and followed by hsp70 and hsp90α at 9 kJ m<sup>-2</sup>. Most of hsp mRNA expression was significantly increased in dose-dependent manners. The expression of hsp10, hsp40 and hsp60 mRNA was not modulated until 15 kJ m<sup>-2</sup>, but rather down-regulated at 18 kJ m<sup>-2</sup> and more, indicating that high dose of UV-B may cause cellular damage in this species. This study will be helpful to obtain a better understanding on molecular mechanisms of protective action against UV-B stress in this species.

**137 Multiple Stressors: The Impact of Fasting and 17β-estradiol on Organ Mass and Gene Expression in Fathead Minnows (*Pimephales promelas*)** A.M. Jessick, Univ of Nebraska Medical Center, Dept of Biology; A.S. Kolok, Univ of Nebraska Medical Center, Environmental,

Agricultural and Occupational Health. The central aim of this study was to examine the levels of expression of estrogen responsive genes in fed and fasting fathead minnows, (*Pimephales promelas*). Two different experiments were conducted in this study. In the first, adult females were either fed or fasted for 7 days. Fasted fish experienced significant reductions in liver and kidney mass, but not in the relative mass of the ovary or the brain. Furthermore, fasting did not significantly influence the hepatic expression of the estrogen-responsive gene vitellogenin. In the second study, male minnows were exposed to fasting, 17 $\beta$ -estradiol (E2) and to both treatments simultaneously. Adult males fasted for 7 days demonstrated significant reductions in liver mass, but gonad and kidney mass remained proportional to body mass, while relative brain mass actually increased during fasting. When exposed to E2, fed males significantly over-express the estrogen-responsive gene VTG, but fasted males exposed to E2 are not significantly different from that in unexposed males. No significant difference was observed in ER- $\alpha$  expression between E2 exposed fed and fasted males compared to unexposed males, but the fed and fasted controls were significantly different. Based upon the results, the interaction between the expression of estrogen responsive genes and fasting differs between males and females. Females maintain normal expression levels of these genes in the face of fasting, whereas the estrogen-induced males demonstrate inappropriate expression of these genes, which appear to be compromised when the fish are fasting.

**138 Population Genetic Monitoring for Toxicant Effect Based on Tolerance Evolution** Y. Tanaka, H. Mano, National Institute for Environmental Studies, Research Center of Environmental Risk; H. Tatsuta, Univ of the Ryukyus, Dept of Ecology and Environmental Science. Evolution of tolerance to a pollutant chemical in a natural population of organisms gives direct evidence of the examined population to have suffered adverse effect by the chemical because the tolerance evolution of a population is solely brought about by selection pressure that removes sensitive individuals or genotypes from the population. Despite the advantage in using the tolerance evolution as a monitoring tool of chemical pollution in the field, such approach is not relevant for a quantitative impact evaluation because observed differences in the tolerance between a contaminated and an uncontaminated site are rarely connected to the ecological risk that burdens the population. Applying the theory of evolutionary ecology and quantitative genetics into the tolerance evolution, we attempted to estimate the selective force that is needed to maintain the observed difference in the tolerance between populations. For this aim, we made a heuristic assumption for the evolutionary analysis of tolerance: the strength of tolerance induced by a genotype to a particular compound is positively associated with fitness cost of the tolerance, and the joint action of selection induced by the cost and selection favoring higher tolerance achieves the optimization of the total fitness that takes into account the fitness gain by the tolerance and the fitness cost due to the tolerance. The fitness gain by the tolerance means reduced toxicity of a specific exposure concentration in comparison to sensitive individuals, and can be quantified by the life table toxicity data. As a case study of such an approach, we detected inter-population differences of the fenvalerate tolerance among natural populations of a water flea (*Daphnia galeata*) in Kasumigaura Lake (Japan) and an agricultural reservoir near to this lake. There was a statistically-significant negative association, among isofemale clones, between tolerance values and intrinsic population growth rates under the null exposure. With the fitness optimization, we derived an estimate of stationary exposure level of the chemical (all compounds that exhibited co-tolerance with this chemical) as 15 ppb, and concluded that the examined population of this species had been suffering the population-level effect that corresponded to 1/4 reduction of the intrinsic population growth rate. The indicated level of the population-level effect may have considerably reduced the probability of persistence of the population.

**139 UV-B Modulates DNA Damage Responses in the Monogonont Rotifer, *Brachionus orientalis*** J. Lee, Hanyang Univ, Dept of Chemistry, Hanyang Univ Graduate School, Dept of Chemistry; J. Rhee, Hanyang Univ; E. Won, Hanyang Univ, Dept of Environ Mar Science; B. Kim, Hanyang Univ, Dept of Chemistry. To understand the effect of UV-B radiation on the rotifer, *Brachionus orientalis*, several parameters including 24-h survival rate, population growth rate, and ROS level were measured after exposure to a wide range of UV-B doses. To check the expression of other important inducible genes such as replication protein A (*RPA*), DNA-dependent protein kinase (*DNA-PK*), *Ku70*, *Ku80*, and heat shock proteins (*hsp*s) after UV-B radiation, we observed dose- and time-dependency at 2 kJ/

m<sup>2</sup>. We also examined 13 *hsp* genes for their roles in the UV-B damaged rotifer. Results showed that UV-B remarkably inhibited the population growth of *B. orientalis*. The level of intracellular reactive oxygen species (ROS) was high at 2 kJ/m<sup>2</sup>, suggesting that 2 kJ/m<sup>2</sup> would already be toxic. This result was supported by other enzymatic activities, such as GSH levels, glutathione peroxidase, glutathione S-transferase, and glutathione reductase. For dose dependency, low doses of UV-B radiation (2, 4, and 6 kJ/m<sup>2</sup>) significantly up-regulated the examined genes (e.g., *RPA*, *DNA-PK*, *Ku70*, and *Ku80*). For the time course study, *RPA* genes showed immediate up-regulation but returned to basal or lower expression levels compared to the control 3 h after UV-B exposure. The *DNA-PK* and *Ku70/80* genes significantly increased, indicating that they may be involved in repairing processes against a low dose of UV-B exposure (2 kJ/m<sup>2</sup>). At the basal level, the *hsp90 $\alpha$ 1* gene showed the highest expression, and followed by *hsp10*, *hsp30*, *hsp60*, and *hsc70*, and *hsp90 $\beta$*  in adults (w/o egg). In eggs, the *hsp10* gene was expressed the highest, and followed by *hsp30*, *hsp27*, *hsp90 $\alpha$ 1*, and *hsp60* genes. In real-time RT-PCR array on rotifer *hsp* genes, low doses of UV-B radiation (2 and 4 kJ/m<sup>2</sup>) showed up-regulation of several *hsp* genes but most of the *hsp* genes showed down-regulation at 8 kJ/m<sup>2</sup> and higher, indicating that significant *Hsp*-mediated cellular damage already occurred at low doses. For the time course study of four *hsp* genes (*hsp20*, *hsp27*, *hsp70*, *hsp90 $\alpha$ 1*), they showed a significant correlation for UV-B radiation (2 kJ/m<sup>2</sup>). In this paper, we demonstrated that UV-B radiation would affect growth retardation with up- or down-regulation of some important genes in DNA replication, repair process, and chaperoning. This finding provides a better understanding of molecular mechanisms involved in UV-B-mediated cellular damage in the rotifer, *B. orientalis*.

**140 The pH Dependence of Natural Organic Matter Sorption to Nanoparticles and Its Ability to Disperse Particles in Aqueous Solutions** P. Mwaanga, Clemson Univ, Environmental Toxicology; E. Carraway, Clemson Univ, Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology. Several studies have demonstrated that natural organic matter (NOM) can reduce toxicity of most toxic chemicals through sorption /complexation processes. In the area of nanoecotoxicology, this has also been demonstrated. The sorption of NOM on to the nanoparticles has further been lauded for particle dispersion and hence lessening the effect of particle aggregation. In this study, the particle dispersion of NOM on both sonicated and nonsonicated TiO<sub>2</sub> nanoparticles at different pH values was examined. The study further examined the sorption of NOM to TiO<sub>2</sub> at the same pH values. The dynamic light scattering (DLS) technique was used to characterize the aggregates. The PALS Zeta potential analyzer was used to estimate surface charge. The total organic carbon was measured by the Total Organic Carbon Analyzer- Shimadzu (TOC-VCPH). The results indicated that the particle dispersion by NOM is pH dependent and is more pronounced at higher pH, but least at pH values close to the point of zero charge (PZC) for the TiO<sub>2</sub> particles. The ability of NOM to disperse nonsonicated particles was found to be mild. As expected, the sorption results showed that the least amount of NOM is sorbed at higher pH, despite the observation that the highest dispersion occurred at higher pH.

**141 Impact of ZnO Nanoparticles on the Dynamic Energy Budgets of the Marine Mussel *Mytilus galloprovincialis*** E.B. Muller, Univ of California, Marine Science Institute; R.M. Nisbet, Univ of California, Ecology, Evolution and Marine Biology; S.K. Hanna, H.S. Lenihan, Univ of California, Bren School of Environmental Science and Management; R.J. Miller, Univ of California, Marine Science Institute. Traditional methods for the analysis of toxicity data are limited in scope, as conclusions drawn from such analyses typically depend on the choice of endpoint, exposure duration and/or laboratory conditions, such as feeding regime. These limitations are overcome with Dynamic Energy Budget (DEB) theory, a powerful tool to integrate the impact of environmental conditions and toxic effects on the production of individual organisms. The results of a DEB based analysis are independent of endpoint and exposure duration. We use DEB theory to analyze the sublethal effects of ZnO nanoparticles on the marine mussel *Mytilus galloprovincialis*. We exposed individuals of different sizes to ZnO nanoparticles (nominal concentrations in the range of 0–2 mg/l) in laboratory-controlled conditions for 3 months. To fully specify the dynamics of toxicant exchange and energy budgets, we measured Zn tissue concentrations, Zn clearance rates, increase in shell length, production of somatic and reproductive biomass, respiration and feeding rates. Because mortality was only significant at the highest exposure level, mussels were exposed to the



full range of sublethal concentrations of ZnO nanoparticles. Biomass production and increase in shell length declined and tissue Zn levels increased with increasing ZnO exposure levels, indicating that our data are amenable to DEB analysis. We will show how the results of this analysis can be used to project the impact of toxicants on the long-term population growth rate.

#### 142 Investigating the Mechanisms Underlying the Effects of Titanium Dioxide Nanoparticles on Two Species of Freshwater Phytoplankton

**K. Kulacki**, Univ of California – Santa Barbara, Ecology, Evolution, and Marine Biology, Univ of California Santa Barbara, Earth Research Institute; S. Bennett, Univ of California Santa Barbara, Bren School of Environmental Science and Management; B. Cardinale, Univ of Michigan, School of Natural Resources & Environment. Titanium dioxide ( $n\text{-TiO}_2$ ) is one of the most widely produced and well-studied types of nanomaterial. Despite this, we lack a basic understanding of how this material impacts the structure and function of natural ecosystems. Here we report the results of a laboratory experiment in which we exposed two of the more common species of freshwater phytoplankton – *Chlamydomonas moewusii* and *Scenedesmus quadricauda* – to increasing concentrations of  $n\text{-TiO}_2$  (0, 1, and 100 mg  $\text{L}^{-1}$ ) to examine how  $n\text{-TiO}_2$  influences algal growth.  $n\text{-TiO}_2$  reduced the growth of *C. moewusii* at 100 mg  $\text{L}^{-1}$ , in part because  $n\text{-TiO}_2$  decreased rates of primary production. There were also signs of physiological stress in this alga, as higher intra-cellular chlorophyll content suggested cells were limited by light. In contrast to the effects of  $n\text{-TiO}_2$  on *C. moewusii*, high concentrations of  $n\text{-TiO}_2$  (100 mg  $\text{L}^{-1}$ ) enhanced the growth of *S. quadricauda*. We could not find clear evidence of a mechanism to explain this growth stimulation, as there were no signs of altered metabolism or physiology that might indicate photo-stimulation. There was no evidence that  $n\text{-TiO}_2$  was degrading organic matter in a way that might release limiting nutrients, nor was there evidence that  $n\text{-TiO}_2$  reduced bacterial metabolism in a way that might release algae from competition. Our study reveals the potential for contrasting effects of  $n\text{-TiO}_2$  on different species of algae. We were able to identify several mechanisms that might underlie the impacts of  $n\text{-TiO}_2$  to *C. moewusii*, and we were able to eliminate several potential mechanisms to explain the stimulatory effects on *S. quadricauda*.

#### 143 Acute and Chronic Toxicity of Nanoparticle and Ionic Forms of Silver to *Daphnia pulex*

**E. Costa**, Wilfrid Laurier Univ; J. McGeer, Wilfrid Laurier Univ, Dept of Biology. The objective of this research is to compare the effect of ionic ( $\text{Ag}^+$ ) and nanoparticle silver (nAg) on acute and chronic uptake and toxicity in *Daphnia pulex*. Exposure solutions were prepared and characterized by solution ultrafiltration (e.g., Ag content in  $< 450\text{nm}$ ,  $< 100\text{nm}$ ,  $< 10\text{nm}$ , and  $< 1\text{nm}$  fractions) and dissolution techniques to assess the content of  $\text{Ag}^+$  and nAg. Acute toxicity tests were standard 48 h exposures to determine lowest observed effect concentrations (LOECs) and 50% effect concentrations (EC50s). The time-course of bioaccumulation was measured (whole organism dry weight basis) at the LOEC concentration for either  $\text{Ag}^+$  or nAg LOECs to assess uptake (48 h) and depuration (48 h). *Daphnia* ( $n = 12$ ) were sampled at time 0, 1, 3, 6, 12, 24, and 48 h in both phases to measure whole-body Ag, Na, and Cl. As well, short-term (3 h) uptake kinetics were also measured via exposure to concentrations ranging from 0 to 1  $\mu\text{g/L}$   $\text{Ag}^+$  or nAg. Chronic toxicity tests were standard 21 d exposures to determine survival (LC50) and reproductive (EC50) effects. The acute EC50 for  $\text{Ag}^+$  (0.78  $\mu\text{g/L} \pm 0.1$  95% CI) and nAg (0.83  $\mu\text{g/L} \pm 0.27$  95% CI) were similar but uptake kinetics differed. Short-term nAg accumulation was linear ( $r^2 = 0.95$ ), indicating a non-carrier (diffusion) uptake and  $\text{Ag}^+$  accumulation was hyperbolic following Michaelis-Menton kinetics ( $r^2 = 0.95$ ), indicating carrier-mediated transport. For the uptake and depuration studies (48 h), *Daphnia* accumulated nAg at a faster rate than  $\text{Ag}^+$  but depurated  $\text{Ag}^+$  faster than nAg. Therefore, the results show different uptake processes for the two forms of silver even though the effect endpoints are similar. Exposure solution characterization by ultrafiltration supported the finding that dissolution of nanoparticles to form ionic silver in solution does not play a significant role in nAg toxicity. This research was supported by the NSERC-NRC-BDC Nanotechnology Initiative.

#### 144 Reproductive Effects and Bioaccumulation in an Estuarine Crustacean, *Americamysis bahia*, Following Chronic Exposure to Multi-walled Carbon Nanotubes

**E.R. Roberts**, Univ of Georgia, Environmental Health Science; M.C. Black, Univ of Georgia, Dept of Environmental Health Science; M.E. DeLorenzo, NOAA, National Ocean Service, Dept of Marine Ecotoxicology. Large amounts of carbon nanotubes are currently being

manufactured, which will lead to an increase of nanomaterials into the environment through accidental spills or known releases. Previous research has used acute toxicity tests to assess the potential toxicity of CNTs to invertebrates and fish. However, little information is available on the toxicity of nanomaterials following chronic exposures of aquatic organisms, particularly in marine and estuarine environments. Here, we report the adverse chronic effects of multi-walled carbon nanotubes (MWCNTs) on 7 day old *Americamysis bahia* (formally *Mysidopsis bahia*), an estuarine crustacean, in a water-only system. Four concentrations were tested (0.1 mg/L to 10 mg/L), assessing mortality, maturation stage, and bioaccumulation of  $^{14}\text{C}$ -MWCNTs as endpoints. Within 24 hours, MWCNTs were visible in the gut tracks of individuals exposed to the 10 mg/L concentration, which was confirmed as bioaccumulation through liquid scintillation counting. No significant mortality was noted at any concentration; however, a decrease in sexually mature individuals was present at the higher exposure concentrations. The percentage of sexually immature individuals increased from 20% of the total population in controls to 60% in organisms exposed to 10 mg/L MWCNTs. The experiment will be repeated to test the sensitivities of *A. bahia* at an earlier (0 day old) and later life stage (14 day old). Although the concentrations used in this experiment were high relative to expected environmental concentrations, continuous exposure of MWCNTs could result in a delayed maturation time of *A. bahia* over time, potentially causing population declines.

#### 145 Using Radiolabels for Investigating the Bioaccumulation and Interaction Effects of $^{14}\text{C}$ -SWNT and $^3\text{H}$ -Ponasterone A in *Leptocheirus plumulosus*

**A.N. Parks**, Duke Univ, Nicholas School of the Environment; P. Schierz, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Pratt School of Engineering; R.M. Burgess, USEPA, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, USEPA; K.T. Ho, L.M. Portis, USEPA, Atlantic Ecology Division; M. Perron, Brown Univ, School of Engineering; M. Pelletier, National Health and Environmental Effects Research Laboratory, USEPA, Dept: ORD NHEERL AED; K. Washburn, Univ of South Carolina, Arnold School of Public Health; G.T. Chandler, Univ of South Carolina, Dept of Environmental Health Sciences; L. Ferguson, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Nicholas School of the Environment, Pratt School of Engineering. The development of single-walled carbon nanotube (SWNT) technologies and products continues to increase, and with this comes greater risk of environmental release through waste discharge and product use. During waste treatment, released SWNT will co-occur with other molecular contaminants such as endocrine disruptors, which may adsorb to the surface of SWNT due to its black carbon like behavior. Therefore, it is important to study the interactions of SWNT with microcontaminants. We have previously assessed the bioaccumulation of pristine SWNT in two benthic estuarine invertebrates (*Ampelisca abdita* and *Americamysis bahia*) via ingestion of sediment and food (algae *Cyclotella sp.* and brine shrimp *Artemia salina*) amended with SWNT. Near infrared fluorescence (NIRF) spectroscopy was used as a sensitive and specific detection method to quantify and characterize SWNT. This study showed uptake of SWNT from amended algae in non-depurated amphipods (35–50  $\mu\text{g/g}$  dry tissue), but the depurated amphipod body burdens were below the detection limit ( $< 1.4$   $\mu\text{g/g}$  dry tissue). Although NIRF spectroscopy provides more detailed SWNT characterization and can be used to analyze environmental samples, radiolabels offer a lower detection limit (0.044  $\mu\text{g/g}$ ) for laboratory-based bioaccumulation studies. To capture this greater sensitivity, a parallel study was performed exposing *Leptocheirus plumulosus* to sediment and food (*Isochrysis galbana*) amended with  $^{14}\text{C}$ -SWNT at nominal concentrations of 10  $\mu\text{g/g}$  and 100  $\mu\text{g/g}$ . After a 28-day exposure, depurated and non-depurated amphipods exposed to 100  $\mu\text{g/g}$  sediment, displayed body burdens (0.3–0.65  $\mu\text{g/g}$  dry tissue and 3–7  $\mu\text{g/g}$  dry tissue, respectively) significantly different from controls. The dual-label, mixed-contaminant system was tested using  $^{14}\text{C}$ -SWNT (100  $\mu\text{g/g}$ ) and the ecdysteroid  $^3\text{H}$ -Ponasterone A (1 ng/g), each individually and in a mixture. A second form of black carbon (coconut charcoal) was used as a positive control. After a 28-day sediment exposure, the bioaccumulation of each contaminant in *Leptocheirus plumulosus* was determined. Results of these experiments will be presented in the context of the relative effects on the bioavailability and bioaccumulation by benthic deposit-feeding organisms of SWNT and microcontaminants present in the environment as mixtures.

**146 Bioaccumulation and Trophic Transfer of Nanoparticles to**

***Leptocheirus plumulosus*** B. Jackson, Dartmouth College, Dept of Earth Sciences; C. Chen, Dartmouth College, Dept of Biological Sciences. The bioaccumulation and trophic transfer of CdSe quantum dots (QDs) and Ag nanoparticles (AgNPs) to *L. plumulosus* was assessed in a series of exposure and toxicity tests conducted in waters of varying salinity. Trophic transfer was investigated by exposing the amphipods to nanoparticles in the presence or absence of the algae, *Isochrysis galbana*. The relative particle size distribution of the nanoparticle in the water column; aggregated, dispersed, dissolved, was assessed by filtration at 0.45 and 0.2  $\mu\text{m}$  and ultra-centrifugation at 3KDa. Animals were exposed to nanoparticles for 96 hrs and allowed to depurate for 24 hrs. Body burden of nanoparticles were assessed by digestion and ICP-MS analysis. Exposures were compared with an equivalent concentration of dissolved metal ion (Cd for the QDs). AgNPs aggregated readily at salinities of 5, 10 and 20‰. Bioaccumulation of AgNPs was greater for 10nm particles than 50nm particles but less than an equivalent concentration of dissolved Ag. No differences in bioaccumulation were observed at higher salinities. Mortality was only observed for dissolved Ag at 5‰. No differences were observed in the presence or absence of algae. QDs remained dispersed (or < 0.45  $\mu\text{m}$ ) at 20‰ salinity. Bioaccumulation of QDs was greater than equivalent dissolved Cd both in the presence and absence of algae. However, mortality was much greater in the presence of algae. Laser ablation ICP-MS was used investigate qualitative differences in QD assimilation between exposure routes.

**147 Multi-generational Impacts of Carbon Nanomaterial Exposure on the Model Organism *Daphnia magna***

D. Arndt, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences, Great Lakes Water Institute; R. Klaper, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences, Great Lakes Water Institute. We examined how carbon nanomaterial core structure and surface chemistry influence the toxicity of these materials to the progeny of an exposed  $F_0$  generation of *Daphnia magna*.  $F_0$  daphnids were exposed to various types of carbon nanomaterials with different core structures and functionalizations. Chronic toxicity was measured on the parent population by evaluating mortality and reproductive parameters over a 21-day period. The neonates produced by the  $F_0$  generation were raised for an additional 21-day period and mortality and reproduction were assessed. This was repeated for the next 3 generations of *Daphnia*. Data indicate that some carbon nanomaterials can have an impact on the reproductive capacity of future generations of daphnids from exposed parents. These results indicate that a single generation exposure may have an impact on future generations of organisms. Future experiments will look at the mechanism by which nanomaterial exposure may impact these subsequent generations of daphnids after a parental exposure, including methylation of DNA in the promoter regions of relevant genes.

**148 Dyes, Old Contaminants that Still Need Toxicological Evaluation**

G.A. Umbuzeiro, UNICAMP, FT; H. Freeman, North Carolina State Univ, College of Textiles. Colours are part of our life. Everything that surrounds us are naturally or artificially colored. Dyes have been used all around the world in textiles, food, hair, plastics, houses, vehicles, cosmetics and other products. In the past they were metal based products. Because of the high toxicity and persistence of metals in the environment they were substituted by organic compounds, that are biodegradable. Thousands of different dyes are produced and in use these days. Because they are visible in very low concentrations, we believed that they would not be present in hazardous amounts. But when dyes from different colours are mixed a brownish to grey colour is obtained, sometimes difficult to be noticed when reaching a water body. In general dyes are very complex organic molecules. The most abundant group are the azodyes, that are produced from aromatic amines. Several aromatic amines has proven to be carcinogenic and are now prohibited. We thought that dyes would be degraded in the biological treatment and only the detection of their former aromatic amines would be enough to evaluate their possible hazard. But recently this paradigm was proved to be wrong, different dye transformation products, such as the PBTAs were found in rivers. They were even more mutagenic than the parental dyes and are colorless. Different dyes as well their transformation products have been found in rivers, sediments, treated effluents, sludges and even in drinking water. Thin layer chromatography, although an old and not sophisticated technique was key for their detection. HPLC methods are now under development to quantify dyes and their transformation products that are present in the environment in ppb to ppt concentrations. The majority of

the detected compounds were not yet tested for ecotoxicity or genotoxicity and other important endpoints. It is now impossible to estimate the risks regarding the exposure of those compounds because no consistent hazard evaluation is available in the literature. Another key information is that the commercial dye products can contain more than 50% of impurities, other dyes and aromatic amines, and are usually sold in unknown surfactants, also not properly evaluated. Dyes, although old contaminants can be considered emergent compounds and should be properly tested before being released into the environment.

**149 First Stages in the Ecological and Human Health Assessment of Aromatic Azo and Benzidine-based Compounds on Canada's Domestic Substances List**

J.R. Hill, A. Okonski, Environment Canada, Government of Canada, Ecological Assessment Division; B. Aikawa, S. Blechinger, M. Gagne, K. Hughes, Health Canada, Government of Canada, Existing Substances Risk Assessment Bureau. In June 2010, the Government of Canada published a Notice of intent to assess the potential health and ecological risks of approximately 350 aromatic azo- and benzidine-based substances on Canada's Domestic Substances List. Substances included in this assessment are among those identified as priorities for action through the Domestic Substances List Categorization process. The aromatic azo- and benzidine-based substances are those which may break down to certain aromatic amines of concern (including benzidines). Some substances within this class or substances similar to those included in this class have been identified by other jurisdictions as a concern due to the potential cleavage of the azo bonds that can lead to the release of aromatic amines that are known or possible carcinogens or genotoxicants. In addition, recent data gathering initiatives have confirmed some of these substances are found in imported consumer products (e.g., clothing, leather products) in Canada. The Government of Canada will apply weight-of-evidence and precaution in decision making. Accordingly, where data are limited and where considered appropriate, assumptions with a reasonable level of conservatism will be applied in the risk assessment. For example, assumptions may be applied regarding the potential for these substances to degrade or metabolize to simple aromatic amines, to migrate or leach from finished products, or to be dermally absorbed from products in contact with the skin. A general scope of the approach will be outlined with focus on chemical clustering based on structural similarities, common functional uses, and physical-chemical properties of these substances. Potential data gaps will be identified and discussed.

**150 Assessing the Bioaccumulation Potential of Substances with Low Bioavailability: Benchmarking a Dispersible Colorant**

M. Bonnell, Environment Canada, New Substances Division, Science and Risk Assessment Directorate; J. Hill, Environment Canada. A general lack of empirical data at the time of categorization of the Canadian DSL (2006) required that, from an ecological standpoint, evidence from quantitative structure-activity relationship (QSAR) models be used to fill data gaps. While this approach was a practical means to accomplishing the prioritization of thousands of chemicals, key ecological hazard properties (i.e., bioaccumulation and ecotoxicity) may be incorrectly predicted for substances with a potentially low to very low bioavailability. We will demonstrate this potential for error using a dispersible colorant and illustrate how its bioavailability and reactivity compares with a benchmark PCB and a solvent dye and how it contrasts with an acid dye. Data from molecular dimensions, physical-chemical properties, metabolism potential, dietary assimilation efficiency, bioconcentration factor (BCF), biomagnification factor (BMF), biota-soil accumulation factor (BSAF) and ecotoxicity are compared and contrasted with the benchmark chemicals. When these lines of reasoning are considered, it can be shown that internally consistent evidence point towards a low bioaccumulation potential of this disperse dye governed principally by low bioavailability to aquatic organisms.

**151 Effect of Photoelectrochemical Chlorination and Conventional Chlorination on Mutagenicity Reduction of CI Disperse Blue 291 Dye**

M.B. Zanoni, UNESP, Analytical Chemistry; G.A. Umbuzeiro, UNICAMP, FT; R.L. Oliveira, G.J. Zucollo, UNESP, Analytical Chemistry. Guilherme Julião Zocolo<sup>1</sup>, Rafael Leite de Oliveira<sup>1</sup>, Gisela de Aragão Umbuzeiro<sup>2</sup>, and Maria Valnice Boldrin Zanoni<sup>1</sup> <sup>1</sup>Electroanalytical Chemistry Laboratory – Dept of Analytical Chemistry – UNESP – Univ of São Paulo State, Brazil <sup>2</sup> Environmental Ecotoxicology and Microbiology Laboratory – LEAL, Faculty of Technology, Univ of Campinas, Limeira, SP, Brazil



**Abstract** The CI disperse Blue 291 dye, 2-[(2-Bromo-4,6-dinitrophenyl)azo]-5-(diethylamino)-4-methoxyacetanilide is a commercial azo dye highly used in textile industry for dyeing of polyester. The present work evaluates the chlorination process effect on dye treatment using photoelectrocatalytic process and conventional chlorination. The removal of CI Disperse Blue 291 dye from wastewater of textile industry was investigated and the generated by products were monitored by using spectrophotometry, chromatographic, organic carbon, mass spectra analysis and mutagenicity assays. The process efficiency was compared with conventional chlorination process commonly adopted in DWTP. CI Disperse Blue 291 dye was almost completely mineralized and its mutagenicity was removed under optimized conditions of photoelectrocatalysis using  $\text{Ti/TiO}_2$ , using both NaCl as sodium sulfate as supporting electrolytic solution in a short time scale treatment (120 min). The results indicate that byproducts generated by photoelectrocatalytic chlorination are free of amine substituent, which seems to be the key for its mutagenicity.

#### 152 The Toxicity of Commercial Dye Disperse Red 1 and His Main Fraction Dye to Estuarine Copepod

M. Artal, Univ of Campinas; A. Albuquerque, Univ of Campinas, Av. Conego Manuel Alves, 220; G. Lotufo, US Army Engineer Research and Development Center; G. Umbuzeiro, Univ of Campinas. Azo dyes are the most abundant and representative class of dyes. Commercial forms of these compounds are mostly a mixture of the main dye, surfactant and other dyes. Components of the commercial dye Disperse Red 1 were separated by TLC (thin-layer chromatography) and the main fraction, corresponding to CI Disperse Red 1 were about 60% of the mixture. We compared the acute toxicity of the main fraction and the commercial dye using the estuarine copepod *Nitokra* sp. Both the commercial dye and the fraction were dispersed with ultrasound sonication for 8 min because they are poorly soluble in salt water. The acute toxicity tests were performed in quadruplicate, 10 organisms per replicate, with marine reconstituted water (salinity 20), photo-period 12-12h at 23°C for 96 hours with adults. We tested 6 concentrations ranging from 0.03 to 30  $\text{mg.L}^{-1}$ , and a negative control, for both samples. After exposure the immobility of organisms was recorded with the aid of a dissecting microscope and the EC50 was calculated by Trimmed Spearman-Kärber method. Sensitivity tests were performed monthly with zinc sulfate as a reference toxicant and the average value is 3.3  $\text{mg.L}^{-1}$  (N=8). Acute median-effect concentrations (EC50, 96h) for Commercial dye Disperse Red 1 and its main dye were virtually identical, 0.13  $\text{mg.L}^{-1}$  and 0.14  $\text{mg.L}^{-1}$ , respectively. If only the main dye was responsible for the toxicity effect of the commercial product, the EC50 for the commercial product would be lower than that observed for the main dye. Therefore we suggest that the toxicity of commercial dye Disperse Red 1 is caused by CI Disperse Red 1 as well as other components in the mixture. The isolated surfactant and the other dyes present in the commercial product are being tested under the same conditions. These results highlight the importance of testing the commercial product and not only the main substance before approving the products for use.

#### 153 Application of Organic Dye Measurements in Airport Stormwater Runoff as Tracers for Assessing Runoff of Aircraft Deicing/Anti-icing Fluids

L. Ferguson, Duke Univ, Dept of Civil and Environmental Engineering; Duke Univ, Nicholas School of the Environment, Pratt School of Engineering, Dept of Civil & Environmental Engineering; S.R. Corsi, US Geological Survey, Water Resources Division. Aircraft deicing and anti-icing fluids are used extensively during winter weather events at airports to minimize risks of ice/snow-related safety incidents. The widespread use of these fluids, which contain a number of potentially hazardous organic contaminants (including glycols, benzotriazoles, surfactants, and colorants) has led to significant concern over their impacts on surface waters and groundwaters in proximity to airports. To date, it has been difficult to trace the contribution of deicers vs. anti-icing fluids (which are more toxic) to stormwater loads of BOD and other markers of organic contaminant concentrations. We have taken advantage of the unique and relatively class-specific dyes used in deicer/anti-icer fluids in order to trace inputs of these two major types of deicing fluids to surface waters. Results indicate that dye identity and concentration are unique between anti-icing and deicing fluids. Tartrazine was a commonly observed "yellow" dye in Type IV anti-icing fluids, while Orange II and Sunset Yellow FCF were the most prevalent dye type in Type I deicing fluids. Measurement of these dyes in surface waters and runoff samples indicates that they can be used as a reliable tracer for deicing and anti-icing fluid inputs to stormwater.

**154 Biodegradation and Toxicity of the Reactive Azo-dye Remazol Red by *Brevibacterium* sp Strain Isolated from Activated Sludge of the Textile Industry** E. Franciscon, Faculty of Technology, State Univ of Campinas, Laboratório de Ecotoxicologia e Microbiologia Ambiental "Prof. Dr. Abílio Lopes"; L.R. Durrant, Faculty of Food Engineering, State University of Campinas, Department of Food Science; Gisela Umbuzeiro, UNICAMP, FT. Azo dyes account for the majority of all dyes produced and have been the most commonly used synthetic dyes in the textile, food, paper making, color paper printing, leather and cosmetic industries. The main azo dye possess the function azo as group chromophoric, are characterized by the presence of one or more azo bond  $-\text{N}=\text{N}-$  on the aromatical systems. Under usual dyeing conditions these dyes don't bind to fibers what results in colored effluents. Dyes removal is desired, not only by esthetic reasons, but also because many azo dyes and their derived products are toxics to aquatic life and mutagenic to humans. Although activated sludge has been extensively used on industries this treatment has not been effective and dyes are adsorbed in the biomass, being later disposed in landfills, contaminating these wide areas. Trying to solve these problems, *Brevibacterium* sp., a bacterial strain isolated from activated sludge of textile industries near Campinas (SP, Brazil) was cultivated with growth medium plus Reactive azo-dye Remazol Red RR (RBN 198) in static conditions. The dye degradation was determined by UV-visible spectrophotometer and confirmed by Total Organic Carbon (TOC) reduction. The degradation products were also characterized by HPLC- MS techniques, and their toxicity measured using *Daphnia similis*. *Brevibacterium* sp. strain degraded completely the dye present in the medium in 24 hs and after 7 days the reduction in TOC was ~70%. The *Daphnia similis* tests were carried out in a 1:4 dilution of the original supernatant concentration, because 100% mortality occurred in the undiluted and 1:2 diluted dye media. Untreated dye showed immobility of 47% at a dilution of 1:4. Samples take from static culture was less toxic, immobility? 13%. The HPLC- MS reveled possible compounds of the RR198 azo dyes biodegradation, in the sample: 4-chloro-N-o-tolyl-1,3,5-triazin-2-amine; sodium 4-aminonaphthalene-2-sulfonate and 3,6-dimethyl-7-(o-tolylidiazetyl) naphthalen-1-amine. The results demonstrate that the static process using a *Brevibacterium* sp. was successful in achieving a complete decolourization and partial degradation of azo dyes. The acute toxicity for *Daphnia similis* after treatment revealed to be lower than untreated dye, indicating the high potential of the bacterium for the use in azo dyes removal.

**155 The Carcinogenic Potential of a Black Dye Commercial Product (BDGP) in Rats Subcutaneously Exposed** R.O. de Lima, Universidade Estadual de Maringá – UEM; G.E. Fontes, Unesp; G.A. Umbuzeiro, UNICAMP, FT; D.F. Salvadori, UNESP. Azo dyes are used and sold as commercial products. They contain, besides surfactants, impurities such as other dyes and aromatic amines. Water contamination by dyes is a matter of concern because they have been found in river waters, sediments, sludges and drinking waters. The components of a black dye commercial product (BDGP), Disperse orange 37, Disperse blue 373 and Disperse violet 93 were tested positive for mutagenicity in the Salmonella microsome assay and were detected in Cristais river in Brazil. The source of the dyes in the river was identified as a textile plant upstream the intake of a Water Treatment Plant. The textile plant effluent showed a high mutagenic effect in the Salmonella assay; potentially carcinogenic activity was also detected in the aberrant crypt foci assay in rats orally treated. Herein, we investigated the carcinogenic potential of the BDGP in distal colon of male Wistar rats. Aberrant crypt foci assay was used to estimate the frequency of preneoplastic lesions. The animals were subcutaneously exposed at doses of 25, 40 or 60  $\text{mg/kg}$  b.w. After 6 weeks no significant effects on animal body weight gain and water and food consumption ( $p > 0.05$ ) were observed. However, significant increase of aberrant crypt foci and aberrant crypts (AC) were found in those rats subcutaneously treated with the highest dose of BDGP. In conclusion, the results demonstrated the carcinogenic potential of BDGP and also highlight that actions must be taken to prevent human exposure to this compound. Toxicokinetics of those compounds are still poorly understood and further studies are required to understand how the dyes can be activated in mammals. Supported by CNPq.

**156 Detection and Spatial Imaging of Heavy Metals in Feathers Using an Electron Probe Microanalyzer** F.R. Wolter, Fayetteville State Univ, Biological Sciences. Using birds as environmental indicator species and analyzing their feathers for accumulated toxic elements is a well-established protocol (Burger 1994). A number of studies have attempted to address



the distribution of external versus internal metal contamination of feathers (Dauwe, et al. 2003; Ek, et al. 2004; Jaspers, et al. 2007). However, traditional chemical assays, such as atomic spectroscopy, condense and consume the entire sample during analysis; thereby, creating two fundamental issues: (1) it is difficult to differentiate findings between bioaccumulation inside and atmospheric deposition on the outside of a feather, and (2) these methods destroy the sample; thus, they are not available for subsequent analyses or for imaging spatial detection. Consequently, this proof of concept study explores the applications of novel avian ecotoxicology research techniques and procedures by using an electron probe microanalyzer (EPMA) to detect, spatially describe, and quantify both the internal and external variation of heavy metals in a single feather. An extensive literature review indicates that this research approach has not been investigated previously. The objectives for the presented portion of this study are two-fold: (1) to determine if and how feathers could be mounted for electron probe microanalyses; (2) to determine if heavy metals may be both detected in a feather sample using wavelength dispersive spectroscopy (WDS) and displayed in detailed images. Primary flight feathers of an adult Carolina Wren (*Thryothorus ludovicianus*) and an adult Red-eyed Vireo (*Vireo olivaceus*) were obtained through the mist netting of live birds known to be nesting and foraging in habitats found within an EPA listed Superfund site in North Carolina. Findings showed that feather samples mounted in an epoxy resin and appropriately polished could be EPMA analyzed. Additionally, using WDS allows for spatial detection imaging of arsenic, mercury, lead, and cadmium. To address challenges related to quantifying amounts of detected elemental x-rays, continued exploration of applying this analytical method applied to feathers is necessary.

**157 Optimization of Stirbar Sorptive Extraction for the Study of Water Accommodated Fractions of Diesel/Biodiesel via Gas Chromatography Mass Spectrometry** R. McCreary, Univ of Texas at El Paso, Dept of Chemistry; W. Lee, Univ of Texas-El Paso. Accidental release of diesel and biodiesel from handling, processing, and consumption is a continued threat for the aquatic environment. Studies have shown that the presence of these fuels can have adverse health effects on a variety of organisms. Due to the continuing demand for diesel and increasing demand for biodiesel, it is imperative that an efficient, effective, and environmentally conscious method be developed to extract and analyze the presence of these compounds in fresh water and sea water accommodated fractions (FWAF & SWAF) so that toxicity of such constituents and concentrations can be addressed. The objective of this study is to implement green chemistry in an analytical method by using stir bar sorptive extraction-thermal desorption-gas chromatography/ mass spectrometry (SBSE-TD-GCMS). Using practically no solvent, SBSE offers great sensitivity for a variety of organic compound at found at very low concentration in aqueous matrices, potentially applicable in monitoring as well as identifying different suppliers and types of oil. Organic compounds—fatty acid methyl esters (in biodiesel) and alkanes (in diesel)—that remain in fresh and sea water will be analyzed. Optimization of this method involved using different methanol concentration and varying stirring time in SBSE process. The results of the optimized conditions and the profiles of water accommodated fractions of these fuels will be presented.

**158 A Gas Chromatography/Triple Quadrupole Mass Spectrometry Based Method for Determination of Hydrophobic Organic Contaminants in Freshwater and Seawater** H. Zhang, National Univ of Singapore, Dept of Civil and Environmental Engineering; S. Bayen, B.C. Kelly, National Univ of Singapore, Dept of Civil and Environmental Engineering. The occurrence of hydrophobic organic contaminants (HOCs) such as polychlorinated biphenyls (PCBs), DDTs and chlordanes in aquatic environments is an important ecological and public health issue due to their capabilities of bioaccumulation and adverse biological effects. Conventional analytical methods using gas chromatography single quadrupole mass spectrometry (GC-MS) is often limited due to high matrix interfaces and relative low selectivity. A potentially more robust approach is to employ gas chromatography triple quadrupole tandem mass spectrometry (GC-MS/MS). Detailed information regarding GC-MS/MS based methods for the analysis of HOCs is scarce. The purpose of this study was to develop GC-MS/MS method for quantifying individual PCBs, organochlorine pesticides (OCPs) and synthetic musk fragrances (SMFs) in freshwater and seawater following solid phase extraction. Target analytes included PCBs with 2-10 chlorine atoms, OCPs such as endosulfan, hexachlorocyclohexanes (HCHs), chlordanes and aldrin, and synthetic nitro and polycyclic musks such as musk ketone and musk xylene, galaxolide (HHCB) and tonalide (AHTN).

Precursor and product ions were chosen according to the results of MS1 full scan and Product Ion scan, respectively. MRM results using different collision energies were compared to obtain optimal collision energy for each MRM transition. Dwell time was optimized using system software. Performance characteristics including linearity, limit of detection (LOD) and limit of quantification (LOQ) were calculated and evaluated. Freshwater and seawater samples, collected at various locations around Singapore were filtered through 1 µm glass fiber filters. Filtrate and filters were spiked with <sup>13</sup>C or deuterated mass-labeled surrogate internal standards. Solid phase extraction with C<sub>18</sub> Empore Disks and sonication extraction were applied to filtrate and filter, respectively, to determine freely dissolved and particulate bound chemical concentrations. A series of spiking experiments were conducted to validate the developed GC-MS/MS method and determine method detection limits (MDLs) of individual target analytes. Observed concentrations and patterns of PCBs, OCPs and SMFs in Singapore's freshwater and marine environments are reported and discussed.

**159 Improved Affinity Extraction of Emerging Contaminants from Water** E. Papastavros, D. Hage, Univ of Nebraska-Lincoln, Dept of Chemistry; D. Snow, Univ of Nebraska-Lincoln, Water Sciences Laboratory, School of Natural Resources; D. Cassada, Univ of Nebraska-Lincoln, Water Sciences Laboratory, School of Natural Resources. Emerging contaminants are chemical compounds that have not traditionally been thought of as pollutants. They comprise a wide range of compounds, including pharmaceuticals, hormones and endocrine disrupting compounds. Emerging contaminants are typically found in surface and groundwater in trace amounts and have agricultural, municipal and industrial sources. They are of concern because their effects on the environment and on human health are not fully understood. A novel, affinity-based extraction method for these compounds is being developed and preliminary results indicate the potential for its use with environmental samples. In this method, a serum protein is immobilized onto a chromatographic support and placed in a column used to selectively bind desired analytes. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) is commonly used for the analysis of environmental samples with solid phase extraction (SPE) being used for the extraction and concentration of analytes. However, more efficient and selective methods of extraction are sought because of the complexity of wastewater matrices. Immunosorbents are other affinity-based extraction methods that have been successful in such applications but require expensive antibodies. Serum proteins such as bovine serum albumin (BSA), which were used in the majority of this work, offer a lower cost alternative that can still bind strongly to various emerging contaminants. The BSA extraction column used in these experiments had only a slightly lower binding capacity than a C<sub>18</sub> column of the same dimensions but had much higher retention for certain pharmaceuticals, hormones and pesticides and could bind to a wide range of such compounds.

**160 A Comparison of Three Sediment Extraction Procedures: Accelerated Solvent Extraction, Microscale Solvent Extraction, and Shaker Extraction** J.R. Thorn, E.S. Barrows, K.P. Cushing, D.M. Schumitz, K.M. McInerney, Battelle, Applied Research and Laboratory Operations; E. Webb, Astrix; C. Cooper, Battelle, Statistics and Information Analysis. Green chemistry or sustainable chemistry involves the use of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. In the analysis of environmental sample matrices for organic contaminants, the benefits of using green chemistry technologies—reduced waste and use of energy and resources—translate into less solvents, reagents, and prep time. EPA and many scientific organizations promote green chemistry through their projects and programs including educational activities and research and development. But how do the results of sample analyses by green chemistry extraction methods compare to results of samples processed using the traditional sample extraction techniques? Four samples of riverine sediment having varying levels of contamination were extracted using three extraction techniques: accelerated solvent extraction (ASE®), EPA Method 3545A; microscale solvent extraction (MSE), EPA Method 3570; and an orbital shaker table procedure. Final extracts from these sediment samples were analyzed by gas chromatography-mass spectroscopy operated in selected ion monitoring mode (GC/MS-SIM) for polynuclear aromatic hydrocarbons (PAHs), both parent PAHs and alkylated compounds, and by GC/MS-SIM for polychlorinated biphenyl (PCB) congeners. Each sample was analyzed in triplicate allowing for evaluation of replicate analyses. Sample analytical results generated by each of the three procedures were used to

assess reproducibility, comparability, sensitivity, and cost. Reproducibility was determined by the relative standard deviation (RSD) of the triplicate analyses using each procedure. Comparability was determined by a statistical comparison of the triplicate analyses using all of the procedures. The impact of differing sample masses employed by each procedure on analytical sensitivity was determined using the achieved laboratory detection limits. To compare sample preparation costs of each procedure, consumption of laboratory solvents and reagents, as well as labor hours were also considered. Our investigation summarizes these factors to present a comparison of ASE and MSE green chemistry methods to traditional sample extraction.

**161 Tracking Hypoxic Conditions via Mo Accumulation: The Influence of N Loading and Local Residence Time** W.S. Boothman, US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory; L. Coiro, M. Abdelrhman, W.G. Nelson, US Environmental Protection Agency. One approach to developing nitrogen (N) criteria for coastal waters is to determine quantitative relationships between N loading and ecological effects, such as hypoxia. Hypoxia may vary significantly within estuaries, making it difficult to document over large spatial and temporal scales. Accumulation of molybdenum (Mo) in surface sediments has been proposed as an indicator of the duration of hypoxia in overlying waters, providing a metric to evaluate the relationship between varying N loads and the occurrence and duration of hypoxic conditions in more than a dozen southeastern New England (USA) estuaries. Nitrogen loads were calculated for each estuary based on watershed land use. Because effects of nitrogen are expected to vary with residence time of the nitrogen within estuaries, N loads were normalized in each estuary for volume and local residence times (LRT) derived from hydrodynamic modeling to account for tidal flushing. Multiple sampling sites were selected within each estuary to span a range of normalized N loading, and surface sediments collected at each site for Mo analysis. A linear relationship between the concentration of Mo in surface sediments and the annual duration of hypoxia (defined as dissolved oxygen concentrations below 2.8 mg/L) was derived for southeastern New England estuaries and used to convert Mo concentrations to average annual duration of hypoxia. This presentation will illustrate the spatial distribution of hypoxia derived from the Mo data and the quantitative relationships between N load, residence time and extent/frequency of hypoxia. By combining these relationships with knowledge of hypoxia tolerance in local or critical species, this approach may be useful to evaluate criteria for nitrogen loading in coastal waters.

**162 A Comparison of Total Nitrogen Measurement Techniques in Surface Fresh Water** A. Jones, Clemson Univ, Institute of Environmental Toxicology; E. Carraway, Clemson Univ, Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology. Total nitrogen (TN) concentrations are of great importance in surface waters because TN is a controlling factor in the total productivity of the system. A major goal has been the replacement of the Kjeldahl nitrogen analysis, which is laborious and generates toxic wastes. The work presented here compares the Kjeldahl method to the alkaline persulfate digestion method, with three different detection methods, and to nitrogen combustion with a silicon diode detector. The three alkaline persulfate digestion methods presented are ion chromatography (IC) with conductivity detection, cadmium reduction with colorimetric detection, and cadmium reduction with IC-conductivity detection. The nitrogen combustion method requires very little sample treatment prior to analysis and is not labor intensive, however the alkaline persulfate digestion methods require some sample treatment prior to analysis. Surface water samples from small, wadeable streams in South Carolina comprise the majority of the analyses and range from 0.1 mg/L TN to 5 mg/L TN (n=200). Regression analysis, analysis of variance, and error analysis are used to assess the performance of the different methods. Results indicate that the alkaline persulfate digestion method yields better recoveries of total nitrogen in surface water samples than nitrogen combustion, but may not be a complete replacement for the Kjeldahl method.

**163 Using Whale Earwax Plugs as a Matrix for Reconstructing Contaminant Profiles in a Marine Ecosystem** E. Robinson, Baylor Univ, The Institute of Earth, Ecological, and Environmental Sciences; S. Trumble, Baylor Univ, Dept of Biology; S. Usenko, Baylor Univ, Dept of Environmental Science. Chemical trends and profiles that have been reconstructed from matrices, such as sediment and ice cores, have provided a wealth of information regarding contaminant behavior and environmental fate, periods of

stress, and climate change. Like all mammals, whales excrete wax into their ear canals; however, whales accumulate their earwax over their lifetime (~20 to 100 yrs) and form layered earwax plugs similar to a sediment core. Therefore, whale earwax plugs may represent a marine matrix capable of recording and archiving a whale's lifetime contaminant profile. An analytical method for identifying historic and current-use pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in lipophilic whale earwax was developed. A method was developed using pressurized liquid extraction, an accelerator solvent extractor (ASE), to extract contaminants from ~0.5 g of each layer of sectioned earwax. In-cell ASE cleanup sorbents, silica gel, alumina oxide basic and acidic, florisil, and celite, were optimized. The contaminants were analyzed using gas chromatography-mass spectrometry with electron capture negative ionization. Results indicated a gray whale's earwax plugs were a viable matrix since pesticide and PCB contaminant profiles were identified in its chronologically layered earwax plugs.

**164 Method Validation and Identification of Halogenated Flame Retardants in Great Lakes Fish** J.J. Pagano, State Univ of New York-Oswego, Environmental Research Center; B. Crimmins, Clarkson Univ; M. Milligan, SUNY Fredonia, Dept of Chemistry; T.M. Holsen, X. Xia, P. Hopke, Clarkson Univ. Industrial chemists have been very busy producing numerous flame retardant chemicals and mixtures to replace polybrominated diphenyl ethers (PBDE) and associated technical mixtures DE-71™, DE-79™, and DE-83™. Several of these replacement halogenated flame retardant compounds have been previously detected in Great Lakes water, sediment, air, and biota. Many of the halogenated flame retardants have yet to be identified in the environment, but are candidates based on their production volume, persistence, bioaccumulative potential and toxicity. Analysis of these legacy, emerging, and reemerging contaminants is complicated by the formation of intermediates and losses during routine sample processing, analysis, and/or environmental degradation. It is unclear if the many of the by-products are impurities during the manufacture or degradation products formed in the environment. As part of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) we analyzed whole fish composite samples for several halogenated flame retardants in the Great Lakes' top predators utilizing both low and high resolution gas chromatographic and mass spectrometric methods.

**165 Analysis of Synthetic Musk Compounds in Great Lakes Fish** M. Milligan, SUNY Fredonia, Dept of Chemistry; B. Moore, SUNY Fredonia; X. Xia, B. Crimmins, P. Hopke, Clarkson Univ; J. Pagano, SUNY Oswego. As part of the USEPA sponsored Great Lakes Fish Monitoring and Surveillance Program (GLFMSP), we are analyzing whole fish composites collected at different sites from all five of the Great Lakes for a suite of contaminants such as Hg, PCBs, PBDEs, and organochlorine pesticides. Recently, we have embarked on full scan MS screening and SIM mode targeted analyses of new and emerging chemicals of concern, such as new-generation brominated flame retardants, industrial chemical intermediates, polysiloxanes, and synthetic musks. In this work, we report on preliminary results of synthetic musk compounds found in Great Lakes Lake Trout fish tissue. Seven synthetic musk compounds were chosen for analysis: galaxolide, tonalide, celestolide, phantolide, traseolide, musk ketone, and musk xylene. Five gram whole fish homogenates were extracted using Accelerated Solvent Extraction (ASE), followed by preliminary clean-up using automated gel permeation chromatography. The synthetic musk compounds were further isolated using a de-activated silica gel column, followed by GC/MS-EI analysis. The pre-dominant musk compounds detected in Great Lakes fish were galaxolide and tonalide, known to be the two highest volume synthetic musk compounds. Total synthetic musk concentrations in whole fish ranged from about 3 to 10 ng/g, with L. Ontario demonstrating the highest concentrations. Additionally, the profiles of these eight compounds in Great Lakes fish are very similar to what has been previously measured in air and dissolved water samples in the Great Lakes.

**166 Current Use Flame Retardants: Chemicals Used in Polyurethane Foam and Their Measurements in Indoor Environments** H.M. Stapleton, Duke Univ, Nicholas School of the Environment; S. Klosterhaus, San Francisco Estuary Institute; A. Keller, P.L. Ferguson, Duke Univ; S. van Bergen, East Bay Municipal Utility District; E. Cooper, Duke Univ; T.F. Webster, Boston Univ School of Public Health; A. Blum, Green Science Policy Institute. Polybrominated diphenyl ethers (PBDEs) were a class of additive flame retardant chemicals used to reduce the flammability of resins and

polymers used in a variety of consumer products, particularly polyurethane foam found in furniture. Due to their persistence, bioaccumulation potential, and potentially toxic, PBDEs have been, or are currently slated for, phase-out in different regions of the world. Despite the phase-out, flammability standards still exist for a number of different products and now other types of halogenated flame retardant formulations are increasingly being used as replacements for PBDEs. Over the past several years our laboratory has analyzed more than 100 polyurethane foam samples collected from both general furniture items (e.g., chairs, couches, etc) and baby products (e.g., nursing pillows, car seats, strollers, sleep positioners, etc.), to identify the most common flame retardants used in these products. Our research indicates that a majority of products containing polyurethane foam, and which are sold in the United States, are treated with several types of chlorinated organophosphate flame retardants (OPFRs). Tris (1,3-dichloroisopropyl) phosphate (TDCPP), is the most common flame retardant detected in foam. One brominated flame retardant mixture known as Firemaster® 550 (FM 550), was also detected in approximately 20% of the products tested. FM 550 is a mixture containing both non-halogenated organophosphates such as triphenyl phosphate (TPP), and iso-aryl phosphate isomers, and two brominated compounds that we refer to as TBB (tetrabromobenzoate) and TBPH (tetrabromophthalate). Two chlorinated organophosphate flame retardants not previously documented in the environment were also identified, one of which is commercially sold as V6 and contains tris (2-chloroethyl) phosphate (TCEP) as an impurity. These current use-flame retardant chemicals have also been detected in indoor dust collected from homes along the eastern US. Levels of these current-use flame retardants in indoor dust are often comparable to the levels of PBDEs, and suggest that human exposure to these new flame retardants is similar to PBDEs.

**167 Emerging Organohalogen Contaminants and Comparative Spatiotemporal Trends and Sources in Herring Gulls From the Great Lakes** R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Great Lakes Institute for Environment, Canadian Wildlife Service; D. Chen, W.A. Gebbink, Carleton Univ, Chemistry; S. Chu, L.T. Gauthier, P. Martin, C.E. Hebert, Science and Technology Branch, Environment Canada; C.D. Weseloh, Canadian Wildlife Service, Environment Canada. Environment Canada's Great Lakes Herring Gull Monitoring Program (GLHGMP) has been ongoing for more than 30 years, where eggs are collected annually from fifteen colony sites. Up until recently, for contaminant monitoring purposes these collected eggs have been used largely for spatiotemporal monitoring of legacy, organochlorine contaminants. However, over the last 4 years, recently collected and archived GLHGMP egg samples have been heavily utilized for screening for priority and previously unknown (to wildlife) pollutants, and subsequently for retrospective examinations of spatiotemporal trends and sources of new substances that may be of health concern to wildlife in the Great Lakes basin. The herring gull has been well-proven to be an excellent biomonitoring species, and although feeds on mainly an aquatic diet, has been shown over the last several decades (depending on the colony in question) and using chemical tracers such as stable carbon and nitrogen isotopes, to be feeding increasingly on terrestrial food sources. As a result, and considering the source of emerging organohalogen contaminants, such dietary changes are being shown to be increasingly useful in elucidating the source of such contaminants to herring gulls. In the present study, we will examine the growing number of bioaccumulative, chlorinated, brominated or fluorinated substances, including flame retardants, surfactants, metabolites and degradation products, being identified in herring gulls (eggs), and the comparative levels, patterns, spatial and temporal trends.

**168 NOAA's Enhanced Great Lakes Mussel Watch Program: Monitoring Contaminant Levels in Mussels at Historic Mussel Watch Sites and Areas of Concern (AOC)** K. Kimbrough, NOAA, Mussel Watch Program. The Mussel Watch Program (MWP) monitors the status and trends of chemical contamination in US coastal waters, including the Great Lakes using bivalve mollusks. In the Great Lakes, zebra and quagga mussels (*Dreissena* sp.) are the sentinel bivalves used for MWP monitoring. In 2009 and 2010 the MWP expanded its program into Areas of Concern (AOCs) in Lakes Erie, Ontario, Huron, Michigan, and Superior. Results will be presented for legacy contaminants, bivalve histopathology, and contaminants of emerging concern (CECs). Specifically, data will be presented that highlight the magnitude of 2009-2010 levels relative to historic data for the nation. A

comparison will also be made between AOCs and ambient MWP sites to characterize the range of concentrations found in this study.

**169 Perfluoroethylcyclohexane Sulfonate and Other Perfluorinated Surfactants in the Great Lakes Aquatic Ecosystem** A.O. De Silva, Environment Canada, Water Science and Technology Directorate; C. Spencer, Environment Canada, Water Science and Technology Directorate; S.R. de Solla, Environment Canada, Science and Technology Branch; R. Letcher, Environment Canada; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, National Water Research Institute; B. Scott, Environment Canada, Water Science and Technology Directorate; S.B. Gewurtz, Environment Canada, Dept of Geography; S. Backus, Environment Canada; P.V. Hodson, Queen's Univ, School of Environmental Studies. Since Giesy and Kannan's seminal report of perfluorooctane sulfonate (PFOS) in biota in the Great Lakes basin in 2001, several studies have focused on researching perfluorinated chemicals (PFCs) in the Great Lakes. Previous research on PFCs has focused on chemicals with aliphatic perfluorocarbon geometry. Here we present data on a relatively unknown cyclic PFC, perfluoroethylcyclohexane sulfonate (PFECCHS). PFECCHS was first highlighted by Howard and Muir as a chemical of commerce that is likely persistent and bioaccumulative. Chemical inventories indicate that PFECCHS was used in North America and Europe as an erosion inhibitor in aircraft hydraulic fluid but other applications may be possible. Here, we measured ng/L levels of PFECCHS in the water from the Great Lakes. Highest concentrations were in L. Michigan and lowest in L. Superior. Lake trout from all the lakes and American eels from L. Ontario indicated consistent PFECCHS levels in trout from L. Ontario and Erie (mean 2.5 ng/g w/w whole body homog.). In eel homog., PFECCHS was 1.1-4.4 ng/g and in liver, 4.2 -39 ng/g, approx. 20x < PFOS, indicating that like other PFCs, PFECCHS has a propensity for liver accumulation. PFC-containing aqueous-film-forming foam (AFFF) for fuel-based fire suppression is one likely source of certain compounds in the Great Lakes. High concentrations of PFOS (ave 2 ppm) were in the plasma of snapping turtles from L. Niapenco, Ontario. L. Niapenco is downstream of an international airport along the Welland River, eventually draining into L. Ont. Spatially resolved sampling of water and benthic inverts here suggested the airport being a dominant PFOS, perhaps due to AFFF usage. PFECCHS showed a similar gradient from the airport, suggesting aircraft hydraulic fluids may indeed be a major application of PFECCHS.

**170 Polychlorinated Biphenyl Congeners in Chicago Air and Lake Michigan Air and Water in 2010** Z. Rodenburg, Univ of Iowa. Polychlorinated biphenyl (PCB) concentrations in air and Lake Michigan water near Chicago declined rapidly following the production ban of Aroclors over 30 years ago. However, since the early 1990s, concentrations of these chemicals have remained relatively constant. During summer of 2010, a field sampling expedition was conducted on Lake Michigan aboard the EPA R/V Lake Guardian approximately 5 km off the coast of Chicago. High-volume air and water samples were collected using Amberlite XAD-2 resin as an adsorption media and quartz fiber filters to collect particles in both phases. Passive air samples using polyurethane foam discs (PAS-PUF) were also collected throughout Chicago and surrounding suburban environments. All samples were extracted via accelerated solvent extraction and analyzed for 209 PCB congeners using tandem mass spectrometry. Quality assurance and control strategies utilized blanks, sample replicates, surrogate standards, internal standard methods and analysis of standard reference materials. Concentration data elicited from each sample was used to calculate instantaneous PCB congener fluxes across the air-water interface at the Lake Michigan location. The results were then used in conjunction with an ongoing regional transport model to predict the effect of specific emissions from the city of Chicago on the magnitude of PCB deposition to Lake Michigan.

**171 Comparison of In Silico and Environmental Measurements for Identifying New, Emerging, and Re-emerging Chemicals of Concern in the Great Lakes** D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, National Water Research Institute; A. De Silva, B. Lee, Environment Canada; P. Howard, SRC, Inc., Environmental Science Center. A wide range of organic chemicals (~350 substances not including structural isomers/congeners) have been reported in Great Lakes air, waters, waste effluents, sediments and biota over the past 15 years (see Klecka et al RECT 2010 for a list of 326 chemicals). The major classes of detected are current use pesticides (CUPs), pharmaceuticals, steroids and



hormones, alkylphenol ethoxylates, antioxidants, synthetic musks, perfluorinated chemicals, polybrominated diphenyl ethers, and other halogenated and organophosphorus flame retardants. Most of the chemicals have been registered since the 1980s under Food and Drug and Pesticide legislation, or on the Toxic Substances Control Act (TSCA) inventory or Canada's Domestic Substances List, in the case of high production volume "industrial chemicals". Given their relatively long term use and recent discovery they might be better termed "re-emerging" rather than "emerging". Their recent identification in environmental media is mainly the result of application of innovative analytical chemistry with a focus on structural analogs of previously identified compounds and application of new techniques such as LC-MS/MS. But this list represents only a small fraction of the possible chemicals that could enter the Great Lakes. There are thought to be more than 30,000 "industrial" organic chemicals in wide commercial use (>1 t/y) as well as about 3000 pharmaceuticals and 1000 CUPs. If their possible degradation products, byproducts, and impurities, are included the list of individual analytes expands enormously. Recently, Howard and Muir (EST 2010;2011) identified 610 organics from a list of 22,263 commercial chemicals and also 364 out of 3193 pharmaceuticals that had potential for persistence and bioaccumulation in the Great Lakes region using U.S EPA EPI Suite software and expert judgment. Of the 610 industrial chemicals, 62% were halogenated and 8% were siloxanes, while most the pharmaceuticals were substituted aromatics. In general, the identified chemicals had molecular structures associated with low rate of biodegradation such as adjacent halogens, cyclics, perfluorinated, cyclic siloxanes, multiple aromatic rings, and relatively high log Kow of 3 to 8. Most of the 350 substances previously measured in the Great Lakes, or in Great Lakes urban effluents, were among the 974 chemicals we identified independently, indicating the value of applying "in silico" approaches provided that structural information is available.

**172 Human Nail and Hair Analysis as Biomarker of Exposure to Perfluoroalkyl Compounds** W. Liu, Dalian Univ of Technology, School of Environmental Science and Technology; Y. Jin, Dalian Univ of Technology. Extensive human exposure to perfluoroalkyl compounds (PFAA) together with their persistence and various toxicities have arisen increasing concern. A noninvasive method would improve exposure assessment for large population, especially the children susceptible to contaminants. The aim of the study was to assess the use of PFAA measurements in human nail and hair as biomarker of exposure to PFAAs. Fingernail, toenail, hair, and blood samples were collected from 28 volunteers. The PFAA concentrations were determined by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Significant correlation was observed between PFAA concentrations in nails, hair, and serum. And nail PFAA level was higher than the serum levels. The accumulation of PFAA in nails and hair, together with their advantages in noninvasive sampling and ability of reflecting long-term exposure made nail and hair PFAA attractive biomarkers of exposure.

**173 Determinants of Serum PFC Levels in Pregnant Women: Results from the Chemicals, Health and Pregnancy (CHiP) Study in Vancouver, Canada** G.M. Webster, Univ of British Columbia, School of Population and Public Health, School of Environmental Health; M. Shoeib, S. Lee, T. Harner, Environment Canada, Atmospheric Science and Technology Directorate; J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine and Pathology; K. Teschke, Univ of British Columbia, School of Population and Public Health. Perfluorinated compounds (PFCs) have been used as stain, grease and water repellents in consumer products for more than 50 years. Despite their ubiquitous presence in human serum, the sources and pathways of PFC exposure are poorly understood. Most studies have relied on simulated exposures, or have considered measured PFC levels in a small number of exposure media (e.g., only diet, or only air and dust). Few studies have examined relationships between measured PFC exposures and serum PFC levels for the same individuals. This study examined associations between a broad range of potential exposures measured at the individual level (i.e., diet, personal characteristics, indoor exposures and PFCs in indoor dust) and serum PFC levels in 152 pregnant women enrolled in the Vancouver (Canada) – based CHiP study. Data on participants' diets, personal characteristics and indoor exposures (e.g., contact with consumer products and time activity patterns) were collected by questionnaire, and PFCs were measured in vacuum cleaner dust from participants' homes. General linear models were used to identify the most important determinants of serum

PFHxS, PFNA, PFOA and PFOS. Final models suggested that pork-based foods, raw fish or shellfish, and food packaging (e.g., microwave and movie theatre popcorn containers) were significant independent predictors of certain PFCs in serum. Parity was strongly and negatively associated with all four PFCs. Ethnicity, time spent in cars, flight time, mattress age, use of stain repellents on carpets, and spot use of stain removers were also predictive of at least one PFC. Dust levels of PFNA and the PFOS-precursor, N-methyl perfluorooctane sulfonamidoethanol (NMeFOSE), were also associated with serum PFNA and PFOS, respectively. These results suggest that both food packaging and bioaccumulation (e.g., into pork, fish and shellfish) contribute to dietary PFC exposures. Associations with pork-based foods are novel and warrant further investigation. The strong negative relationships with parity, suggesting maternal excretion across the placenta and/or through breastfeeding, underline concerns about fetal and infant exposures to PFCs during critical phases of development. Associations of serum PFCs with time spent in cars and airplanes, as well as with stain repellents used on carpets are also novel, and raise questions about the use of PFCs and their precursors in vehicle and airplane interiors, and in post-market carpet care liquids.

**174 Human Detoxification of Perfluorinated Compounds** D.A. Birkholz, ALS Laboratory Group, Research and Toxicology; S. Genuis, Univ of Alberta, Clinical Associate Professor Faculty of Medicine. There has been no proven method thus far to accelerate the clearance of potentially toxic perfluorinated compounds (PFCs) in humans. PFCs are a family of commonly used synthetic compounds with many applications including repelling oil and stains on furniture, clothing, carpets, and food packaging, as well as in the manufacturing of polytetrafluoroethylene (e.g., Teflon) – a non-stick surfacing often used in cookware. Some PFCs remain persistent within the environment due to their inherent chemical stability and are very slowly eliminated from the human body due, in part, to enterohepatic re-circulation. Exposure to PFCs is widespread and some sub-populations, living in proximity to, or working in fluorochemical manufacturing plants, are highly contaminated. PFC bioaccumulation has become an increasing public health concern as emerging evidence suggests reproductive toxicity, neurotoxicity, hepatotoxicity, and that some PFCs are considered to be likely human carcinogens. A case history is presented where an individual with high concentrations of PFCs in serum provided a) sweat samples after use of sauna; and b) stool samples before and after oral administration of each of two bile acid sequestrants – cholestyramine (CSM) and saponin compounds (SPCs). Stool samples before and after use of a cation-exchange zeolite compound were also examined. PFCs found in serum were not detected in substantial quantities in sweat, nor in stool prior to treatment. Minimal amounts of PFOA, but no other PFCs were detected in stool after SPC use; minimal amounts of PFOS, but not other PFCs were detected in stool after zeolite use. All PFCs congeners found in serum were detected in stool after CSM use. Serum levels of all PFCs subsequently declined after regular use of CSM. Further study is required, but this presentation suggests that CSM therapy may facilitate gastrointestinal elimination of some PFCs from the human body.

**175 Legacy and Novel Brominated Flame Retardants in Indoor Dust from India: Characteristics and Implications** G. Devanathan, Ehime Univ, Center for Marine Environmental Studies (CMES); T. Isobe, Ehime Univ, Senior Research Fellow Center (SRFC); A. Subramanian, Ehime Univ, Center for Marine Environmental Studies; N. Kajiwarra, National Institute for Environmental Studies, Research Center for Material Cycles and Waste Management; K. Asante, Ehime Univ, Center for Marine Environmental Studies; G. Suzuki, National Institute for Environmental Studies, Research Center for Material Cycles and Waste Management; S. Takahashi, S. Tanabe, Ehime Univ, Center for Marine Environmental Studies. Brominated flame retardants (BFRs) including polybrominated biphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are a group of chemicals which have been widely used in various consumer products to avoid fire accidents. Unfortunately, these chemicals can migrate from products to environment and ultimately reach humans. Concerns have risen as BFRs share similar properties with well-known toxic chemicals such as dioxins and polychlorinated biphenyls (PCBs). As a result, the production and use of these chemicals have been banned/regulated in Europe, North America and parts of Asia but most developing countries are yet to start such actions due to the availability of limited baseline dataset on these chemical contaminants. Elevated levels of BFRs were reported in air and dust from indoors than outdoor

environments and fears stated as we spend most time indoors. Therefore, the present study focused on elucidating the contamination status of BFRs and assessing human exposure through dust samples collected from different microenvironments including e-waste recycling buildings, commercial premises and residential areas in India. Significantly higher ( $p < 0.05$ ) levels of contaminants such as PCBs, PBDEs, 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE) (median: 7100, 48000, 65000 and 120 ng/g dust, respectively) were found in dust samples from e-waste recycling locations than commercial (median: 23, 460, 220, and 67 ng/g dust, respectively) and residential buildings (median: 13, 1000, 48 and 15 ng/g dust, respectively), suggesting that a major emission source of PCBs and BFRs is the crude e-waste recycling/dismantling activities in India. Levels of HBCDs were relatively low in dust from e-waste recycling buildings (median: 8 ng/g) than residential dust (median: 30 ng/g) indicating the wide use of HBCDs in textiles and other household materials than electrical and electronics products in India. Estimated daily intakes of BFRs via dust ingestion were much higher for toddlers than adults, particularly population involved in e-waste recycling activities are of great concern. More detailed studies of pollution sources and pathways are needed in order to reduce the exposure levels.

**176 First Assessment of Human Exposure to Indoor Organic Contaminants in Pakistan** N. Ali, Antwerp Univ, Pharmaceutical Sciences, Toxicology Centre; N.V. Eede, A. Dirtu, H. Neels, A. Covaci, Univ of Antwerp, Toxicology Centre. Ingestion of indoor dust has been recently acknowledged as an important of exposure to indoor organic contaminants. Here, we investigated the presence of polybrominated diphenyl ethers (PBDEs), novel brominated flame retardants (NBFRs), organophosphates esters (OPEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) in indoor dust from rural homes and mosques in Gujrat, Pakistan. Dust samples were collected from homes living room floors ( $n = 31$ ) and from mosque hall ( $n = 12$ ). PBDEs, NBFRs, PCBs and OCPs were quantified using a GC-ECNI/MS method, while a GC-EI/MS method was employed for the determination of OPEs. Low concentrations were observed for most of the investigated contaminants. Hexachlorocyclohexanes (HCHs) and DDTs were the most prevalent OCPs with median concentrations 0.7 ng/g and 22 ng/g, respectively. PBDEs were only minor constituents in the investigated samples and the most important contributor was BDE 209 (median 40 ng/g). For NBFRs decabromodiphenyl ethane (DBDPE) (median 15 ng/g) was highest contributor followed by 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) (median 3 ng/g). OPEs were present in higher levels than other contaminants with tri-(2-butoxyethyl)-phosphate (TBEP) and tri-phenyl-phosphate (TPP) having medians of 66 ng/g and 110 ng/g, respectively. Using log transformed data; a two-sample t-test was applied to study correlation between homes and mosques dust. PBDEs and NBFRs showed a significant positive correlation ( $p < 0.05$ ), which indicates similar source of emission for BFRs in indoor environment. Surprisingly, levels of HCHs, DDTs and PCBs Congener CB 153 showed a positive correlation ( $p < 0.05$ ) for both homes and mosques dust samples, indicating that a plausible source of contamination could be dust brought in with shoes. For OPEs, TBEP, TnBP, and TPP showed positive correlation ( $p < 0.05$ ) suggesting similar sources of contamination in indoor dust. Over all the results show that the profile of homes and mosque indoor dust were not very different. One way ANOVA followed by Dunnett's post hoc test showed that the levels of investigated analytes were significantly different and lower than Romania, Belgium and New Zealand dust samples, indicating the variable use of these chemicals in different societies. To author's knowledge, this is the first study to document the presence of indoor organic contaminants in Pakistani dust.

**177 US Toddlers Exposure to PBDEs** H.M. Stapleton, Duke Univ, Nicholas School of the Environment; S. Eagle, Duke Univ; A. Sjödin, Center for Disease Control and Prevention; T.F. Webster, Boston Univ School of Public Health. Polybrominated diphenyl ethers (PBDEs) are a class of persistent and bioaccumulative flame retardants that are known to be associated with adverse effects on thyroid regulation and neurodevelopment. While a large research effort has focused on adult exposure to PBDEs, less is known about children's exposure and body burdens. In order to better understand factors affecting children's exposure, we conducted a study which sought to determine if body burdens of PBDEs were significantly associated with house dust and/or PBDE residues on hand wipes collected from the children, and to determine if body burdens were associated with gender,

age, race, and education level of the parents. Blood samples, hand wipes and house dust were collected from children between the ages of 12–36 months and who resided in the central North Carolina area between 2009–2010. A short questionnaire was administered by a research associate which collected information on the child's physical and behavioral characteristics, information on breast feeding, and information on the parents race and education levels. PBDEs were detected in every serum sample and the most abundant congeners were those associated with the PentaBDE mixture. Concentrations ranged from 5.2 to 745 ng/g lipid with a geometric mean value of 46.7 ng/g lipid. When PBDE levels were separated into tertiles by age of the child, PBDE levels were significantly higher ( $p < 0.05$ ) in the oldest age group ( $>24$  months) relative to the youngest age group ( $\sim 12$ –18 months). Significant associations were also observed between PBDE levels and race and maternal education level, with higher levels observed in African Americans relative to Caucasians, and lower PBDE levels observed with higher maternal education levels. In addition, significant correlations were observed between the serum levels of BDE 47 and residues of BDE 47 measured on hand wipes ( $r = 0.60$ ;  $p < 0.0001$ ) and with BDE 47 levels in house dust ( $r = 0.40$ ;  $p < 0.001$ ). No associations were observed between PBDE levels and duration of breast feeding as an infant. These data suggest that children's exposure to PBDEs from house dust appears to be a leading exposure pathway in toddlers, and not diet or breast milk exposure. Minorities and families with lower educational levels are also at higher risk to exposure to this class of contaminants.

**178 Occurrence of Metabolites of Organophosphate Flame Retardants and Plasticizers in Human Urine** N. Van den Eede, Univ of Antwerp, Pharmaceutical Sciences; A.C. Dirtu, Toxicological Centre, Univ of Antwerp, Dept of Pharmaceutical Sciences; E. Dirinck, P. Jorens, Univ Hospital of Antwerp; H. Neels, Univ of Antwerp, Pharmaceutical Sciences; A. Covaci, Univ of Antwerp. Organophosphate esters (OPEs) are used as flame retardants and plasticizers in a wide range of consumer products. Because of their increased use in the last decade, they are now highly abundant in the indoor environment. Human exposure to OPEs would occur *via* indoor air and indoor dust, both in which increased levels have been reported. Exposure to these chemicals may therefore be high and needs to be monitored. OPEs are metabolized in the liver to dialkyl phosphates or diaryl phosphates (DAP), depending on the structure of the parent compound. These DAPs can be traced in human urine and can serve to monitor human exposure to OPEs. The aim of this study was to determine urinary concentrations of DAPs in obese persons who were monitored before and after a reducing diet or bariatric surgery. Six DAPs were quantified, namely bis(1-chloro-2-propyl) phosphate (BCPP), bis(2-butoxyethyl) phosphate (BBEP), bis(2-chloroethyl) phosphate (BCEP), diphenyl phosphate (DPP), bis(1,3-dichloropropyl) phosphate (BDCPP) and di-(*iso/n*-)butyl phosphate (DBP) which are metabolites of the corresponding triester OPEs. Weak anion exchange solid phase extraction was used to extract DAPs from urine, followed by their separation by liquid chromatography on a Kinetex HILIC 150 x 2.1 mm x 2.6  $\mu$ m column. Analysis was performed by tandem mass spectrometry in electrospray negative ionization mode. DAPs were quantified on four deuterium-labeled internal standards using multiple reaction monitoring. DAP concentrations were normalized to creatinine levels to compensate for the urine volume. The resulting data were analyzed using non-parametric statistical tests for relations between the levels of DAPs and gender or age group. Time dependent relations were investigated for concentrations of DAPs before the surgery/diet ( $n=95$ ) with results of 3 months after the surgery/diet ( $n=55$ ) and 6 months after the surgery/diet ( $n=39$ ). The creatinine normalized levels were also compared to results from Germany and the United States. Further, the profiles and concentrations of DAPs in urine were compared with the levels and profiles of OPEs in Belgian and European indoor air and dust, which are the main exposure routes of humans to OPEs.

**179 Predictors of TDCPP Metabolite in Office-Worker Urine** C.C. Carignan, Boston Univ School of Public Health, Dept of Environmental Health; E. Cooper, Duke Univ, Environmental Sciences and Policy; D.J. Watkins, Boston Univ School of Public Health, Dept of Environmental Health; A.J. Fraser, Harvard Univ, Dept of Medicine; W. Heiger-Bernays, M.D. McClean, Boston Univ School of Public Health, Dept of Environmental Health; H.M. Stapleton, Duke Univ, Nicholas School of the Environment; T.F. Webster, Boston Univ School of Public Health, Dept of Environmental Health. Tris(1,3-dichloro-2-propyl) phosphate (TDCPP)

was removed from use in children's pajamas over three decades ago for health reasons, however, is currently used as a flame retardant replacement for PentaBDE in polyurethane foam. Our aims were to characterize office worker exposure to TDCPP by measuring the urinary metabolite bis(1,3-dichloro-2-propyl) phosphate (BDCPP) using a newly developed bioassay and examine possible predictors of exposure. Urine collected from Boston office workers ( $n=29$ ) was analyzed for BDCPP using LC/MS-MS and adjusted for specific gravity. Dust samples were analyzed for TDCPP using GC/MS. We detected BDCPP in 100% of urine samples at concentrations ranging from 62 to 1,760 pg/ml and a geometric mean (GM) of 408 pg/ml. We detected TDCPP in 99% of the dust samples with the highest concentrations in vehicles (GM=12,500 ng/g) as compared to offices (GM=6,060 ng/g) and homes (GM=4,210 ng/g in main living area; GM=1,400 ng/g in bedroom). TDCPP concentrations in dust were not associated with vehicle age (1-13 years) suggesting use in vehicles over many years. Based on the scatterplots, we identified a positive but non-significant trend between urinary BDCPP and TDCPP in office dust ( $r=0.24$ ,  $p=0.21$ ) that was not observed for dust from the home or vehicle. Removal of two influential points strengthened this association ( $r=0.45$ ,  $p=0.02$ ), suggesting that TDCPP in offices may contribute to office worker exposure. Independent of dust levels, average urinary BDCPP was 71% lower in workers from a newly constructed building ( $p=0.003$ ), suggesting that exposure may be affected by other building characteristics. Additionally, handwashing was associated with reductions in urinary BDCPP concentration ( $p=0.095$ ). Controlling for handwashing, workers with offices in a newly constructed building had average BDCPP urine concentrations 68% lower than workers with offices in older buildings ( $p=0.01$ ).

**180 Environmental Cancer Epidemiological Case Study Gibraltar** H. Sanderson, Aarhus Univ, Environmental Science, National Environmental Research, Dept of Policy Analysis; P. Fauser, Aarhus Univ, Environmental Science. This environmental epidemiological study assessed the relative cancer risk and the role of environmental factors herein in Gibraltar compared to the rest of the EU. The study was commissioned by the Gibraltar government in 2008. The study conducted a weight-of-evidence analysis on credible and available environmental data relative to the reported cancer registry data. Air quality parameters and TBT in the drinking water supply, from desalinated sea water to tap water will be shown as special case-studies. The main cancers types comprising the majority of cancers will be reviewed in light of the environmental exposures. The study found that the cancer incidence rates are within the normal ranges of the rest of the EU. The levels of carcinogens in Gibraltar ambient air are within the normal ranges of what is found in the air in cities in the rest EU and within the guideline values of the EU. The study also uncovered significant uncertainties in relation to assignment of carcinogenicity to endocrine disrupting compounds, which have not so far been reflected in the regulatory appreciation of e.g., TBT carcinogenicity. The study results was presented to the government of Gibraltar earlier this year.

**181 Carcinogenic and Non-carcinogenic Health Risks Associated with Former Manufactured Gas Plants (MGP)** R. DeHate, GEI Consultants, Inc., Senior Risk Manager; D. Murray, B. Conte, GEI Consultants, Inc. Regulatory agencies are focused on assessing the potential for soil vapor intrusion (SVI) to adversely affect indoor air and the risk posed to occupants of residential and commercial properties overlying and surrounding former MGPs. This study evaluated the potential for SVI at 10 commercial buildings and 26 single family and multi-family residential properties overlying and/or adjacent to three former MGPs in Long Island, New York. The findings were compared to federal and state background data sets. The results did not identify evidence of MGP-related soil vapor intrusion from any of the 36 sites regardless of depth to water table or proximity to MGP source tar or dissolved phase plumes. In addition, risks were calculated based on maximum and mean concentrations for benzene, toluene, ethylbenzene, and xylenes measured in ambient air, soil vapor, and indoor air samples. These chemicals were selected based on frequency of detection within the data sets. Hazard Indices for non-carcinogenic risk were calculated using the study results and the mean, maximum and 95<sup>th</sup> percentile concentrations from background data bases. Carcinogenic risks associated with benzene were calculated using both the measured mean and maximum study results and the mean, maximum and 95<sup>th</sup> percentile concentrations from background data bases. Hazard Indices for exposures near MGPs were less than 1 or were comparable to the regulatory mean and maximum background levels.

Cancer risks for residential exposures near MGPs ranged from  $1.93 \times 10^{-6}$  to  $3.68 \times 10^{-5}$ . However, background residential benzene exposure not related to former MGP sites ranged from  $9.9 \times 10^{-6}$  to  $3.59 \times 10^{-3}$ . Cancer risks from exposure to benzene concentrations near MGPs were equivalent to or less than cancer risks estimated for a background residence in the northeast United States. No increased health risks were associated with occupied residential or commercial properties overlying or surrounding MGPs.

**182 Risk Factors to Blood Dioxin Concentrations in Vietnamese Citizens Living Near Agent Orange Hot Spots at Da Nang and Bien Hoa, Vietnam** D.T. Pham, Univ of Wyoming, Dept of Zoology and Physiology (3166); H.M. Nguyen, Office of the National Steering Committee 33; T. Boivin, Hatfield Consultants; H.L. Bergman, Univ of Wyoming, Dept of Zoology & Physiology. Agent Orange was one of a number of defoliants used by the US military in Vietnam from 1961-1971; this herbicide was contaminated with dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD). There are a number of major dioxin "hot spots" resulting from the use, distribution and spillage of herbicides at former US military bases in Vietnam. Recent studies at the former Da Nang and Bien Hoa airbases, completed by Hatfield Consultants and the Vietnamese Ministry of Natural Resources and Environment (MONRE), have found very high TCDD concentrations in soils, sediments, fish, human blood and human breast milk, with concentrations in all media greatly exceeding international standards. Elevated dioxin concentrations in the food chain are likely sources of human dietary exposure, posing a very serious health risk for the Vietnamese population living near these hot spot sites. Human health effects are now well documented for US military veterans and include prostate cancer, leukemia and Type II diabetes among other disease syndromes in adults. Possibly even more worrisome for the continuing exposure of the human population in Vietnam, physical and mental birth defects in children have been linked to TCDD exposure in utero and via breast milk. Based on measurements of blood dioxin and results of epidemiological surveys conducted at Da Nang and Bien Hoa by Hatfield and MONRE, we completed multiple linear regressions to assess importance of several risk factors contributing to blood dioxin levels. Important factors assessed included age, living or working at the contaminated sites, consumption of contaminated foods and lifestyle factors. Results of these and related analyses will be presented.

**184 Assessment of Indoor Air Impacts From Naphthalene Associated With Vapor Intrusion at MGP Sites** R. DeHate, GEI Consultants, Inc., Senior Risk Manager; B. Skelly, GEI Consultants, Inc. Naphthalene is a constituent of coal tar and often a contaminant found at former Manufactured Gas Plant (MGP) sites. Development at these sites has resulted in residential and commercial areas with potential exposures from vapor intrusion adversely affecting indoor air of residences and buildings. Naphthalene is routinely analyzed in soil vapor intrusion assessments for properties overlying and surrounding former MGP sites. The United States Environmental Protection Agency and the State of California have both proposed a draft unit risk factor for naphthalene. Naphthalene exposure from vapor intrusion may be a public health risk. The purpose of this study was to evaluate three sites located in the northeast United States to determine the frequency of naphthalene detection in indoor air. A total of 79 properties were included in the study. A total of 546 indoor air samples were analyzed for naphthalene by EPA Method TO-14A or TO-15. The properties were sampled on 161 occasions and some properties were sampled up to 14 times. At residential properties indoor air samples were collected in basements and crawl spaces, and in occupied areas such as living rooms and bedrooms. At commercial properties indoor air samples were collected in basements, sump rooms, storage closets and common areas such as lobbies, classrooms, and offices. The number of samples for each property ranged from three to twenty-eight depending on the size of the building, with an average number of four samples collected from each location. A typical sample collection included indoor air testing in the basement area, living room (with a duplicate sample) and a bedroom. These data were then used to evaluate exposure and assess public health risks. Naphthalene concentrations ranged from 0.26 to 51 microgram per cubic meter of air. Only 3 of the 546 indoor air samples detected naphthalene above the ninety-fifth percentile background value of 12 microgram per cubic meter of air. These 3 detections could be explained by sources of naphthalene not associated with vapor intrusion. Naphthalene was detected in less than 1% of the indoor samples. Naphthalene vapor intrusion impacting indoor air was not a public health risk among the 79 properties built on or near the former MGP sites.



**185 Advantages and Complexities of Tissue-Based Cleanup Goals** K. Godtfredsen, T. Deshler, Windward Environmental LLC; S. Replinger, Windward Environmental, LLC, Environmental Scientist. Recently, the concept of developing tissue-based cleanup goals has been receiving more attention. Although it makes sense to link cleanup goals as directly as possible to the remedial action objective of interest (e.g., reducing risks to seafood consumers), caution needs to be exercised in the development and application of tissue-based goals at sediment sites. Tissue-based goals, coupled with post-remedy monitoring, are best seen in the light of risk communication, which serves an important public benefit. For a number of reasons, however, tissue-based goals should not be used as preliminary remedial goals. Specifically, concentrations of bioaccumulative chemicals in fish, crab, and clam populations can be highly variable as a result of numerous environmental factors, many of which are difficult to predict, much less control. In addition, the derivation of "background" and risk-based goals for tissue must be carefully considered, particularly for "market basket" seafood ingestion scenarios. This presentation will acknowledge the allure of developing tissue-based cleanup goals but also outline key uncertainties and complexities at sediment sites.

**186 Utility of Polytopic Vector Analysis (PVA) as a Tool for Differentiating Background Concentrations of Metals from Site-Related Metals Concentrations** S. Huntley, ERM, Sediments and Watershed Integrated Management (SWiM). Polytopic vector analysis (PVA) is a multivariate statistical procedure originally developed within the geologic sciences, primarily for oil exploration, but in recent years has been employed for fingerprinting a variety of environmental contaminants, most notably polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs). PVA differs from more traditional statistical fingerprinting tools such as principal components analysis (PCA) in that it is an "unmixing" model that (1) identifies the number of source end members in the system, (2) quantifies the chemical composition (e.g., fingerprint) of each end member, and (3) determines the relative contribution of each end member to each sample. For sites that are potentially contaminated with metals, PVA may be a powerful tool for differentiating background metals concentrations from site-related metals concentrations. Results are discussed for several site metals datasets in the context of complicating factors including (1) contributions from both natural and anthropogenic background, (2) potentially different geologic lithologies occurring in varying proportions in different samples, and (3) multiple onsite or offsite sources that cannot be explained by either natural or anthropogenic background.

**187 Potential Impacts of Climate Change on the Ecology of Dengue and its Mosquito Vector the Asian Tiger Mosquito (*Aedes albopictus*)** R. Erickson, Texas Tech Univ, Dept of Environmental Toxicology, TIEHH/Texas Tech Univ, graduate student; K. Hayhoe, Texas Tech Univ, Dept of Political Science; S. Presley, Texas Tech Univ, Dept of Environmental Toxicology; K. Long, L. Allen, Texas Tech Univ, Dept of Mathematics and Statistics; S. Cox, Texas Tech Univ, Dept of Environmental Toxicology. Shifts in temperature and precipitation patterns caused by global climate change may have profound impacts on the ecology of certain infectious diseases. We examine the potential impacts of climate change on the transmission and maintenance dynamics of dengue, a resurging mosquito-vectored infectious disease. In particular, we project changes in dengue season length in three cities: Atlanta, GA; Chicago, IL; and Lubbock, TX. These cities are located on the edges of the range of the Asian tiger mosquito within the United States. We use a disease model that explicitly incorporates mosquito population dynamics and high-resolution climate projections. Based on projected changes under the SRES A1fi (higher) and B1 (lower) emission scenarios, as simulated by 4 global climate models, we found that projected warming shortened mosquito lifespan, which in turn decreased the potential dengue season. These results illustrate the difficulty in predicting how climate change may alter complex systems.

**188 The Effects of Suspended Sediment on Two Strains of Walleye (*Sander vitreus*) Eggs and Fingerlings** B.C. Suedel, US Army Engineer Research and Development Center, CEERD-EP-R, US Army Engineer Research and Development Center, Environmental Laboratory, Waterways Experiment Station EP-R; C.H. Lutz, J.U. Clarke, C.G. Doug, US Army Engineer Research and Development Center. Restrictions on dredging operations via environmental windows are hindering dredging operations in the Great Lakes and other areas of the United States. Sediment resuspension

is often cited as a concern leading to restricted dredging schedules, and is exemplified by walleye (*Sander vitreus*) in western Lake Erie. To address this concern, research was conducted to determine whether suspended sediments affect walleye eggs and fingerlings, mimicking sediment resuspension during dredging operations. In a series of laboratory experiments, hatchery collected northern and southern strain walleye eggs and fingerlings were exposed to multiple levels of suspended sediment collected from western Lake Erie. Newly laid eggs and 45-60 day old fingerlings from two different hatcheries were exposed separately in the Fish Larvae and Egg Exposure System (FLEES) at the US Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, MS. Target concentrations of suspended sediment were 0, 100, 250 and 500 mg/L to mimic suspended sediment concentrations near dredging operations; concentrations were maintained in a flow-through system for three days. After exposure, the eggs were transferred to hatching cups and allowed to hatch. Larvae hatched from exposed eggs and fingerlings were visually inspected for defects, documented, and preserved. The FLEES maintained consistent NTU levels in the exposure chambers during each experiment. Data indicated no overt effects of suspended sediment on either egg hatch success or fingerling survival for either of the northern and southern strains. Gross anatomy and morphology analyses of exposed larvae and fish fry yielded no observable effects. These effects data will better inform the setting of environmental windows for this species in Lake Erie.

**189 Evaluation of Ongoing Versus Legacy Sources Contributing to Elevated Bioaccumulatives in Hepatopancreas of Dungeness Crabs From Victoria Harbour, BC** W.R. Hovel, Weston Solutions, Inc., Anchor QEA; M. Larsen, B. Lamoureux, Anchor QEA; G. Watson, Transport Canada; E. Shankie, Public Works and Government Services Canada; D. Kettlewell, SNC-Lavalin Environment; P. Allard, C. Mackintosh, Azimuth Consulting Group Inc. A Dungeness crab hepatopancreas consumption advisory is currently in effect in Victoria Harbour, BC as a consequence of elevated concentrations PCBs, dioxins, and furans within crabs. Human health risk assessment has indicated potential risks to consumers who frequently consume whole crab as a result of elevated PCBs and dioxin/furan concentrations in the crab hepatopancreas. In addition to localized areas of legacy sediment contamination, elevated concentrations of bioaccumulatives have been measured at some stormwater outfalls; however, the relative contribution of ongoing waterborne sources versus legacy sediment sources to the Victoria Harbour food web is unknown. For purposes of long-term management of Victoria Harbour sediment, this study was designed to evaluate the relative contribution of ongoing sources to the crab tissues such that the need and extent of sediment remediation and risk management decisions can be further evaluated. Water, sediment, and multiple organisms from Victoria Harbour were collected and analyzed for PCBs and dioxin/furans. To assess data prior to food web modeling, data were analyzed for spatial and temporal trends, PCB and dioxin/furan composition patterns including identification of key risk drivers in crab, and relationships among media. PCBs were measured in surface water during both sampling events, with the highest concentrations (2.4 ng/L) during the wet weather (December) event, at multiple locations, suggesting contribution from ongoing sources. Sediment PCB concentrations decreased towards the harbour mouth. Sediment PCB composition patterns were similar throughout the harbour with one exception; higher concentrations of the less chlorinated congeners were measured in one localized area. In biota, spatial patterns in PCBs varied by species; no spatial variability in crab hepatopancreas PCB concentrations or composition patterns was observed. Greater spatial variation in bioaccumulative concentrations was measured in shiner perch. Veneridae family and *Macoma nasuta* clam PCB concentrations demonstrated downward trends towards the harbour mouth, and were significantly correlated with sediment and water PCBs, indicating the potential contribution from both sources. Food web bioaccumulation modeling, which includes species-specific spatial averaging considerations, is underway to further evaluate contribution from different sources. Results will be discussed in the context of the risk management strategy.

**190 An Integrated Evaluation of Methods and Approaches for Assessing Bioavailability of Contaminants in Sediments** N. Bonnevie, ARCADIS, Risk Assessment and Ecological Services; D. Rigg, J. Gravenmier, M. Beauchemin, T. Iannuzzi, B. DeShields, P. Doody, ARCADIS. Decisions regarding the management of contaminated sediments need to consider the potential risks from exposure to chemicals, which are controlled by

various biological, chemical and physical factors internal and external to the ecosystem including the bioavailability of the chemicals in the sediments. Approaches for assessing bioavailability on a site-specific basis are inconsistent and too often decisions regarding risks and ultimately remediation are based on generic information or the total concentration of a contaminant(s) in sediments. This can result in an overestimation of risk from particular chemicals, leading to unnecessary remediation. In addition, for those sites where an assessment of bioavailability is performed, future conditions of the site are sometimes not adequately considered. This is becoming an increasing concern given the present focus on habitat/ecosystem restoration as part of or following remediation. In recent years a variety of sampling and analytical tools—both empirical and mechanistic—have been developed to evaluate the bioavailability of contaminants in sediments, helping to focus the investigation on those contaminants or exposure pathways that are most critical and providing a more realistic evaluation of risks. Although some of these methods are still not standardized (e.g., in situ water column samplers, use of in situ SPMDs, analysis of black carbon, analysis of pore water through SPME), in many instances small and inexpensive additions to a sampling program (e.g., adding pore water, total organic carbon, grain size, AVS/SEM, or other measures) can provide valuable data for evaluating factors that influence bioavailability. In this presentation, we will discuss recent developments in the field of bioavailability and their potential implications on both the evaluation of risks and on decisions regarding the need for or extent of remediation. We will describe key advantages and disadvantages of the more popular approaches, such as bioaccumulation models and normalization techniques and consider the increased evolution and utilization of sampling tools focused on understanding bioavailability. Case examples will be used as appropriate to illustrate key issues and considerations. In addition, we will discuss and provide examples regarding the need to consider potential future conditions in an ecosystem as part of the bioavailability assessment and, ultimately, the remedy selection process.

**191 Atlantic RBCA Version 3 and Tier I Ecological Standards** M. Poirier, Atlantic PIRI (Partnership in Risk Based Corrective Action); R. Mroz, Environment Canada. Atlantic Partnership in Risk Based Correction Action (RBCA) Implementation (Atlantic PIRI) is the multi-stakeholder committee who develops and assists in the ongoing delivery of RBCA in the four Atlantic Provinces. PIRI has a mandate to establish risk-based approaches to manage petroleum contaminated properties in Atlantic Canada. In 1999, Atlantic PIRI adapted the RBCA process, originally developed in the United States, to conditions typically present in Atlantic Canada. Atlantic RBCA has been used as the process for the development of Tier I and Tier II petroleum hydrocarbon assessment/remediation standards protective of human health. The Atlantic RBCA process is supported by two main components: 1) the regulatory-endorsed philosophy of risk assessment, risk management and a tiered approach to remediation; and 2) a technical tool kit (composed of a software model, supporting technical guidance and applicable provincial legislation, regulations, and policy guidance). Since 2007, Atlantic PIRI has also been developing Atlantic RBCA Tier I standards for the protection of ecological receptors with the goal of updating and revising existing ecological receptor screening documentation. A Task Group of scientific subject matter experts from across Canada was formed and developed, adapted and validated ecological based petroleum hydrocarbon criteria for soil, groundwater, surface water and sediment using best available scientific modeling tools. Subsequent sediment toxicity testing validated the modeled predictions. Included with the recent release is a scientific rationale document describing how these standards were adapted and developed. In 2011, the RBCA Tool Kit for Atlantic Canada, Version 3 software and User Guidance were released. Online technical training for users of Atlantic RBCA and updates to ecological receptor screening documentation were also released as key components of Atlantic RBCA implementation in the region. Highlights of Atlantic RBCA Version 3 will be presented including a focused presentation of the scientific rationale used to develop ecological based Tier I standards for petroleum hydrocarbons.

**192 Monitoring the Efficacy and Potential Environmental Effects of In Situ Remediation** C. Menzie, Exponent, Inc., Exponent, Principal; U. Ghosh, Univ of Maryland Baltimore County, Civil & Environmental Engineering. Activated carbon has been used in a number of pilot studies to remediate sediments contaminated with hydrophobic chemicals such as polychlorinated biphenyls (PCBs), methyl mercury, pesticides, and polycyclic aromatic hydrocarbons (PAHs). The efficacy of remediation has

been evaluated by measures of accumulation into passive sampling devices, pore-water measurements, bioaccumulation into organisms exposed to sediments taken to the laboratory, and bioaccumulation into organisms in the field. Results show that these measures provide various perspectives on the risk-reduction potential of in situ remediation. At the scales of pilot studies an important field factor is the "edge effect" that occurs because the pilot study scale is often considerably smaller than the site. For this reason, monitoring needs to be able to recognize the existence and confounding effect of this factor. The presence of edge effects makes some measures of efficacy more reliable than others. The edge effect will diminish as the scale of treatment increases. Modeling also provides a tool for assessing the potential influence of in situ treatment on food-web transfer of the contaminants. A critical aspect of in situ monitoring is the assessment of the potential effects of amendments on the biological communities. Current work involves a combination of laboratory toxicity studies, field community studies, and re-colonization studies. Effects need to be judged within a relative risk framework where the potentially adverse effects of in situ treatment are compared to the potential impacts of alternative remediation approaches. To that end, the EPA's Sediment Management Guidance has been adapted as a framework for comparing the ecological and other costs and benefits of alternative remedial approaches. This paper presents the results of several completed and ongoing in situ treatment projects involving amendment with activated carbon.

**193 Measures of Remedy Effectiveness for the Ottawa River Environmental Dredging Project** M.A. Mills, USEPA, National Risk Management Research Laboratory; R. Brenner, USEPA Office of Research and Development, National Risk Management Research Laboratory; J.M. Lazorchak, USEPA, Office of Research and Development, National Exposure Research Laboratory, Molecular Ecology Research Branch; K. Fritz, USEPA, Office of Research and Development, National Exposure Research Laboratory; J. Meier, USEPA, National Exposure Research Laboratory – SEE Program; A. Mucha, S. Cieniawski, USEPA, Great Lakes National Program Office; D. Walters, US Geological Survey. Contaminated sediments pose risks to human health and the environment. Managing these risks generally involves three options, dredging, capping, and monitored natural recovery. Developing measures (or metrics) of the near- and long-term effectiveness of these remedies is an active area of research within USEPA and other research organizations. We provide an overview of multiple lines of evidence being developed at Ottawa River, a direct tributary of Lake Erie in Toledo, OH. Environmental dredging under the Great Lakes Legacy Act authority was conducted in 2010 in the lower five miles of river near its mouth. ORD in collaboration with GLNPO, USGS, OEPA, and NOAA are conducting a study to identify appropriate and effective metrics and tools to monitor the effectiveness of the remediation and recovery of the system. Specific areas on the river were targeted for removal to various depths depending on remedial objectives for selected contaminants (primarily PCBs, PAHs, and lead). The lines of evidence include physical, chemical, and biological measures that evaluate the recovery on various temporal and spatial scales. Results presented are for pre-, during- and intermediate post-dredging and include data on fish and invertebrate body burden PCB/PAH concentrations, bullhead blood and liver DNA damage, an invertebrate Index of Biotic integrity, passive samplers, as well as other chemical and physical indicators.

**194 Development of Sediment Remediation Effectiveness Guidance for the Great Lakes Legacy Act Program** A.P. Mucha, US Environmental Protection Agency, Great Lakes National Program Office; M.A. Mills, USEPA, National Risk Management Research Laboratory; S. Cieniawski, USEPA, Great Lakes National Program Office. The Great Lakes Legacy Act is a contaminated sediment management program focused on Great Lakes Areas of Concern. To better assess whether the Great Lakes Legacy Act remedial actions are effective in meeting its remedial goals and improving the health of the aquatic ecosystem, a guidance document is being developed for use at Legacy sites. Building upon several completed assessments at Legacy remediation sites that have already taken place, the guidance document seeks to provide a decision-making approach, rather than a compendium of methods. While the guidance is specific to Legacy Act sites, the document benefitted greatly from a multi-Agency workgroup that reviewed, critiqued, and helped shape the guidance. Key findings, the approach recommended, and the process used to develop the guidance will be presented, along with case studies for sites where this assessment approach has been implemented.

**195 Interactions Among the Biology of American Eel (*Anguilla rostrata*), Exposure to Dioxin-like Compounds, Embryotoxicity, and Recruitment** J.M. Casselman, Queen's Univ, Biology; R.S. Brown, Queen's Univ, Dept of Chemistry; J.D. Byer, Queen's Univ; P.V. Hodson, Queen's Univ, School of Environmental Studies; M. Alae, Environment Canada; C.M. Couillard, Fisheries and Oceans Canada, Institut Maurice Lamontagne; M. Lebeuf, Fisheries and Oceans Canada, Maurice Lamontagne Institute; N. Bols, Univ of Waterloo, Dept of Biology. The American eel (*Anguilla rostrata*) is an endangered species in Ontario. Since the 1980s, recruitment of juveniles to L. Ontario declined exponentially, large yellow eels disappeared, and the commercial fishery was closed. One possible cause is the embryotoxicity of dioxin-like compounds (DLCs) that eels accumulate during their growth phase in L. Ontario. Eels accumulate DLCs to the same extent as lake trout (*Salvelinus namaycush*), and trout reproductive failure has been tied to DLC accumulation, among other factors. The eel is semelparous, reproducing once after a 6-mo migration from L. Ontario to the Sargasso Sea, during which feeding stops, tissues are catabolized to sustain migration and oocyte maturation, and lipid stores (20-30% ww) are transferred to oocytes. Eels are also panmictic; eggs and leptocephali are distributed randomly by currents to estuaries and rivers along the east coast of N. America, as far north as Greenland. This phase is initiated by lipids derived maternally. Thus, embryotoxicity and lower recruitment may result from lipid-soluble DLCs. Upon transformation to glass eels and entry to the St. Lawrence R., eels begin a 5–15 y migration up to Cornwall, ON, arriving as juveniles at a dam where a counting ladder provides an index of recruitment. After passing the dam, the eels reside in the upper St. Lawrence R. and L. Ontario for up to 25 y, before maturing sexually and returning to the Sargasso to spawn. This extended life cycle means that eels recruiting to L. Ontario in the 1980s–90s were spawned from parents that integrated the contaminant history of L. Ontario from the 1960s–70s, the period of highest contamination. The extended life cycle of eels, their trophic status, and their high lipid content predict a high sensitivity to DLCs, which are persistent, bioaccumulative, and highly toxic to fish embryos.

**196 Spatial Trends of Legacy Persistent Organic Pollutants in Eels** J.D. Byer, Queen's Univ; M. Alae, Environment Canada; R.S. Brown, Queen's Univ, Dept of Chemistry; M. Lebeuf, Fisheries and Oceans Canada; S. Backus, M. Keir, G. Pacepavicius, Environment Canada; S. Trotter, Fisheries and Oceans Canada; P.V. Hodson, Queen's Univ, School of Environmental Studies. World-wide, freshwater eel (*Anguilla* sp.) populations have undergone severe decline. Persistent organic pollutants (POPs) are currently under consideration as one of many possible causes, related to a reduction in embryo viability following exposure to several classes of POPs. Legacy POPs such as polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans are ubiquitous in the environment and the focus of this study. American eel (*Anguilla rostrata*) were collected between 2007–2009 from seven locations in Canada and one in the United States (Hudson River, NY). European eel (*Anguilla anguilla*) from one location in Belgium served as a positive control to assess the current risk of POPs toxicity to eels. Concentrations of individual POPs classes varied greatly between locations, but the general concentration order was PCBs > OCPs > PBDEs >> PCNs > PCDD/Fs for all locations. In eels collected at Canadian locations, the mean ΣPCBs ranged from 26.2 to 403 ng/g ww, and the ΣPBDEs ranged between 2.29 and 23.6 ng/g ww. There was an apparent gradient from west to east across eastern Canada, with higher concentrations occurring in eels collected in Lake Ontario (western most sampling site), most likely related to higher levels of urbanization and industrialization. The ΣPCNs varied between 18.1 and 354 pg/g ww for all locations. The Canadian samples showed the same west to east gradient in concentration. The Belgian and US samples had similar PCN concentrations to the Lake Ontario eels. PCDD/Fs, dl-PCBs, and PCNs that had available toxic equivalent factors in the literature, were used to calculate 2,3,7,8-TCDD toxic equivalence (TEQs) for fish. The geometric mean for total TEQs ranged from 0.77 to 3.59 pg TEQ/g ww for Canadian collection sites, and 4.44 and 8.18 pg TEQ/g ww for the US and Belgian locations, respectively. The estimated no observable effects threshold for lake trout is 5 pg TEQ/g ww. If eels have similar sensitivity to TCDD as lake trout, the current risk of embryo toxicity from POPs is moderate-low.

**197 Temporal Trends (1988–2008) of Chemical Contamination in American Eel Captured in Lake Ontario** M. Lebeuf, Fisheries and Oceans

Canada, Maurice Lamontagne Institute; J. Byer, Queen's Univ; M. Alae, Environment Canada; S. Trotter, M. Raach, Fisheries and Oceans Canada; S.R. Brown, J.M. Casselman, P.V. Hodson, Queen's Univ. American eels (*Anguilla rostrata*) that grow to maturity in Lake Ontario are known to be highly contaminated with persistent organic pollutants (POPs). This raised the question of the possible role of chemical contamination in the decline of eel recruitment to Lake Ontario in the 1980–90's. However, information on temporal trends of POPs in Lake Ontario eels is lacking. This study aims to provide temporal trend data for legacy POPs including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs). Polybrominated diphenyl ethers (PBDEs), a class of chemicals more recently regulated in Canada, were also analysed in eels. The temporal trend study was based on three groups of eels captured in Lake Ontario in 1988, 1998 and 2008. Each group of eels was comprised of ten females, averaging 20 years of age, collected between June and July. Each eel was homogenized prior to being analysed. Mean lipid content in the 2008 eel homogenates was significantly lower than in 1988 and 1998. A similar trend was observed in legacy POPs where the contamination was significantly lower in 2008, up to 3 fold, than in previous years. Lipid normalisation did not change the observed temporal trends. Toxic equivalents (TEQs) calculated from PCDD/F, non-ortho and mono-ortho PCB concentration data, using fish toxic equivalency factors, revealed the same trends. The results did not show the decrease of POPs contamination generally reported in sediments and biota between 1988 and 1998 in Lake Ontario. Even more surprising, the same trend was observed for PBDE contamination among the three groups of eels. Most temporal trend studies of PBDEs in biota report an increase until about 2000 followed by a steady state or a slow decrease. In summary, concentrations of all the organohalogen chemicals investigated were not different in eels collected in 1988 and 1998 but were significantly higher than in eels collected in 2008. Results of isotopic ratios  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  indicated that eels collected in 2008 are at a lower trophic level compared to 1988 and 1998 eels. These unexpected temporal trends and difference in trophic level suggest that although the three groups of eels were collected in Lake Ontario, they may have grown in different areas of the lake or tributaries.

**198 Perfluorinated Surfactants in American Eels** A.O. De Silva, Environment Canada, Water Science and Technology Directorate; S. Backus, Environment Canada; P.V. Hodson, Queen's Univ, School of Environmental Studies. Globally, freshwater eel populations have declined. In Ontario, the American eel (*Anguilla rostrata*) is classified as an endangered species due to a severe decline in recruitment to Lake Ontario. It has been hypothesized that chemical contamination may be a factor in the decline. As part of a larger group evaluating the persistent organic pollutants burden in eels, we measured perfluorinated compounds (PFCs) in American eels, collected between 2007–2009 collected at several locations in Canada and the US, and for comparison, European eels (*Anguilla anguilla*) from Belgium. Analytes consisted of perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSAs) including a newly detected cyclic perfluorosulfonate. Analysis by LC-MS/MS indicated that the dominant PFC congener was perfluorooctane sulfonate (PFOS), comprising 70–85% of the sum of PFCAs and PFSAs. In whole body homogenates, the sum of PFCAs and PFSAs ranged from 40 to 160 ng/g wet weight with the exception of eels from 3 locations which were less contaminated (< 20 ng/g sum). In those 3 locations, PFOS constituted < 50% of the perfluorinated acid burden. Concentrations in eels from the St. Lawrence River were comparable to those from a site in Belgium. Liver concentrations were also measured since it has been previously established that PFSAs and PFCAs preferentially accumulate in serum and liver. Wet weight concentrations of PFCAs and PFSAs were 2 to 3 x higher in liver compared to whole body homogenate, similar to previous observations in trout. The concentrations observed in eels from Lake Ontario and the St. Lawrence River were approximately 2x higher than in other top predators from the same location, lake trout and walleye.

**199 Dioxin-like Contaminants May No Longer Be a Risk to the American Eel (*Anguilla rostrata*) in Lake Ontario** S.J. Kennedy, Queen's Univ, graduate student; J.D. Byer, Queen's Univ; M. Alae, Environment Canada; R.S. Brown, Queen's Univ, Dept of Chemistry; J.M. Casselman, Queen's Univ, Biology; P.V. Hodson, Queen's Univ, School of Environmental Studies. Starting in the 1980s, recruitment of American eel (*Anguilla rostrata*) to Lake Ontario declined by 99%, resulting in the closure of the eel fishery in Ontario. The decline followed peak contamination of predatory fish species with persistent organic pollutants and recruitment failure of lake



trout associated with high tissue concentrations of dioxin-like compounds. To determine if dioxin-like compounds might also explain the eel recruitment problem, we tested the toxicity of eel extracts to embryos of Japanese medaka (*Oryzias latipes*). Sexually maturing, large yellow and silver eels were sampled in 2007 and 2008 from five locations in eastern Canada, including Lake Ontario, and from the Hudson River (US) and Canal Dessel-Schoten (Belgium) as positive controls. Medaka eggs were injected immediately after fertilization with 1 or 10 nL of eel extract, and scored for signs of toxicity after 11 d. Only extracts from Canal Dessel-Schoten eels caused effects consistent with dioxin-like toxicity in Japanese medaka embryos, indicating that dioxin-like contamination may no longer be a problem in Lake Ontario. However, this conclusion is tentative because medaka are somewhat less sensitive to dioxins than lake trout, and significant mortalities and reduced hatching among treatments at the highest exposure doses suggest that other co-extracted, non-dioxin-like contaminants may affect embryo survival. Compared to the 1980s, the lower concentrations of dioxin-like compounds in sexually-maturing eels implies that recruitment of juvenile eels to L. Ontario should be increasing. This is consistent with a small, but growing number of juveniles climbing the St. Lawrence River eel ladders at Cornwall, Ontario from 2003 to 2009.

#### **200 Embryotoxic Potential of Persistent Organic Pollutants Accumulated by American Eels (*Anguilla rostrata*) from Lake Ontario Between 1988 and 2008**

**C. Rigaud**, Université du Québec À Rimouski; C.M. Couillard, Fisheries and Oceans; J. Pellerin, Université du Québec À Rimouski; B. Legare, Fisheries and Oceans; P.V. Hodson, Queen's Univ; J.D. Byer, Environment Canada. Since the early 1980's, the St. Lawrence River American eel (*Anguilla rostrata*) population is undergoing a dramatic decline associated with recruitment failure, possibly related to the embryotoxic potential of persistent halogenated hydrocarbons (PHHs) which are accumulated in eel tissues and transferred to the eggs. To test this hypothesis, organic extracts were prepared from American eels captured in Lake Ontario in 1988, 1998 and 2008. Extracts from 5 individuals eels per sampling year were concentrated into triolein and then injected into embryos (N=35 per eel extract) of a marine model fish, the mummichog (*Fundulus heteroclitus*), at 0 day post-fertilization (DPF). Embryos were exposed to 10 EEQ/g (Eel Equivalent per gram of embryo). Survival, growth, malformations, hatching success, cytochrome P4501A induction (EROD activity) and behaviour of *Fundulus* larvae were evaluated at 16 DPF. While survival and hatching success were not impacted by any eel extract, a significant increase in the prevalence of craniofacial deformities as well as a significant reduction in growth and prey capture ability were observed with extracts from 1988 and 1998 eels. All extracts significantly induced EROD activity with lower induction for 2008 extracts compared to the older ones. Our results suggest that, during the 80's and the 90's, PHHs accumulated in American eel tissues have reached concentrations potentially toxic to their offsprings. Experiments with lower charges (0.5 to 10 EEQ/g), evaluation of interspecies sensitivity to PHHs and histological analyses (cerebral apoptosis, brain and retinal damages) are underway.

#### **201 Effects of Contaminants on the Reproductive Success of American Eels**

**A. Carey**, K. Oliveira, Univ of Massachusetts Dartmouth, Dept of Biology; A. Roe, US Fish and Wildlife Service; W. Hable, Univ of Massachusetts Dartmouth, Dept of Biology. The recent decline in worldwide populations of Anguillid eels is thought to be caused by pollution, overfishing, climate change, and/or habitat destruction. American eels, *Anguilla rostrata*, are known to inhabit contaminated rivers for the majority of their lives until they migrate to the ocean to spawn. Due to their unique catadromous life cycle, mature adults are not accessible for study and little is known about the effects of contaminants on fertilization and larval development. Using methods similar to the maturation of *Anguilla japonica*, *A. rostrata* males and females were artificially matured with weekly hormone injections. Using these procedures, our objectives were to (1) examine the maternal transfer of environmental contaminants to the eggs, (2) examine the effects of contaminant load on the fertility of females, and (3) examine the effects of polychlorinated biphenyls (PCBs) on the reproductive success of male eels. To examine the maternal transfer of contaminants to the eggs, ovulated eggs and muscle tissue are being analyzed for PCBs, dioxins and furans, pesticides, polybrominated diphenyl ethers, and metals. Results, to date, provide important baseline data and will facilitate development of a model for predicting parental contaminant contribution to offspring. To examine the effects of contaminant load on the reproductive potential of females,

time to full maturation, ovulation, and fertilization success were evaluated and will be compared to the contaminant load in eggs. To examine the effect of PCBs on male gametogenesis, fertilization, embryogenesis and early larval development, males were co-injected with 2 different concentrations (1.0 or 10 µg/fish) of PCBs. Fertilization success and embryogenesis were assessed 2–4 hours post fertilization (PF), 24 hours PF, and 48 hours PF. Preliminary results show that males injected with 10 µg of PCBs exhibited lower reproductive success and produced embryos with disrupted early embryonic development. Fertilization success was significantly lower in embryos fertilized with sperm from males injected with 10 µg PCBs compared to the controls. Our results will shed light on whether environmental contaminants are contributing to the decline of *A. rostrata*. These findings will be important for future conservation efforts and the management of the American eel, an ecologically and commercially important species.

#### **202 Impacts of Habitat Contamination on the Health of Declining American and European Eel Populations**

**P. Couture**, INRS, Centre Eau Terre Environnement; C.M. Couillard, Fisheries and Oceans Canada, Institut Maurice-Lamontagne; L. Bernatchez, Université Laval, Département de Biologie; P.G. Campbell, INRS-Eau, Terre et Environnement, INRS-ETE, INRS, Centre Eau Terre Environnement; F. Pierron, CNRS; M. Baudrimont, Université Bordeaux 1, Station marine d'Arcachon; H. Budzinski, Université Bordeaux 1, Institut des Sciences Moléculaires; P. Elie, CEMA-GREF, Unité Ecosystèmes Estuariens et Poissons Migrateurs Amphihalins; S. Dufour, Muséum National d'Histoire Naturelle, UMR BOREA. The European and American eels are two economically, ecologically and culturally important fish species currently considered in decline and, in the case of the European eel, threatened by extinction. The role of pollution in the decline of these fish is still largely unknown. This new research project is a joint initiative of researchers from Québec and France. Its general objective is to examine the relationships between pollution, both inorganic and organic, and the health of Atlantic eels in the Gironde and the St. Lawrence Estuaries. Indeed, contaminants released in water by urban, industrial, mining and agricultural activities accumulate in yellow eels during their long phase of somatic growth and could affect their growth rate as well as cause tumors and lesions. Furthermore, during their reproductive migration, silver eels mobilize their energy reserves to fuel migration and for gonad maturation. Accumulated contaminants could then be released massively and cause toxicity in the adult or be transferred to embryos. The first objective of this study is therefore to test the hypothesis that contaminants exert a selective pressure on eel populations that will translate into differences in genotypic composition between populations from clean and contaminated sites, using 454 pyro-sequencing to identify SNP markers. The second objective is to understand the influence of the contamination of habitats where yellow eels grow on energy accumulation, metabolic capacities, growth, tissue and cell damage and histopathology, using a range of molecular, biochemical and physiological approaches including the development of a microarray. The third objective is to evaluate the effects of contaminant mobilisation and redistribution on health indicators (oxidative damage, histopathology, genotoxicity), migratory capacities (metabolism, condition) and gonad maturation (hormonal and histological changes, gonad contamination and follicular atresia) of female silver eels. Ultimately, this research, conducted in partnership with key government agencies, will improve our capacity to favour the restoration of Atlantic eel populations, through the development of informed management policies."

**203 Reducing Uncertainty in Risk Assessment: Lessons Learned from Studying Transfer of Organic Contaminants from the Abiotic to the Biotic Compartment** H. Selck, Roskilde Univ, Dept of Environmental, Social and Spatial Change; D. Salvito, Research Institute for Fragrance Materials, RIFM; V. Forbes, Univ of Nebraska Lincoln, School of Biological Sciences. Potential hazards and risks of new and existing chemicals are assessed by regulatory agencies based on their P (persistence) and B (bioaccumulation) and T (toxicity) potential. Being able to accurately identifying PBT substances is therefore key to protect human health and the environment. The focus of this presentation will be on the importance of environmental and physiological factors for affecting bioaccumulation including biotransformation of organic contaminants in the aquatic environment and thus for their persistence. Examples will include results from a range of experiments performed in collaboration with the Research Inst. for Fragrance Materials (RIFM) using different sediment-associated fragrance materials (FMs), sediment-dwelling deposit-feeders, and different sediment-organic contents. Here we focus on: 1. effects of varying organic matter content in the sediment; 2. impact of species dependent biotransformation differences for the fate (accumulation, biotransformation) of sediment-associated FMs. These studies support the conclusion that the materials presented in the examples are not PBTs. Due to their feeding strategy (ingest massive volumes of sediment) and a digestive system optimized to extract organic material from sediment, deposit-feeders may increase the amount of contaminants taken up from the gut. However, since these organisms, generally, also show high biotransformation capacities which evidently will reduce the body-burden (BB) of organic contaminants, focusing solely on BB will significantly underestimate accumulation and thus increase the uncertainty of e.g., BAF. Since benthic communities may play an important role both in the remobilization of sediment-associated organic compounds but also by affecting their fate (bioturbation, biotransformation) this will also have implications for the way we evaluate the persistence of organic contaminants in risk assessment. To improve reliability of current risk assessment of PBT substances we need to focus future research on factors affecting P and B and focus on the transfer from the abiotic to the lower trophic level as this generates the basis for further B assessment.

**204 Exploiting Relationships Between Metrics of Biouptake in Fish to Improve Laboratory to Field Extrapolation** D. Mackay, Trent Univ; F. Gobas, Simon Fraser Univ; J. Arnot, Univ of Toronto Scarborough. Biouptake of organic chemicals in a variety of organisms is of continuing scientific and regulatory interest. Metrics such as BCF, BAF, BME, BSAF and TMF are widely measured, some in laboratory tests and some by field monitoring. Considerable progress is being made in determining and interpreting these quantities. Applying simple and well-accepted mass balance models of uptake and clearance for fish shows that these metrics are closely related thus providing an opportunity, for example, to extrapolate more accurately from a laboratory BCF to a field BAF and a field BME. These relationships are discussed and it is shown that a combination of laboratory and field data with a model can provide a powerful quantitative description of the biouptake phenomena for both scientific and regulatory purposes. Further, the relatively high degree of reproducibility achievable in laboratory tests could be better exploited for deducing field conditions and effects by some simple modifications to existing test protocols. Suggestions are made for such modifications, for gathering more accurate and relevant chemical properties and physiological parameters of the test organisms and for designing test and sampling programs using tentative advance uptake/clearance models. This could lead to an improved overall biouptake test strategy and to improved risk assessments.

**205 Passive Dosing, Passive Sampling and Benchmarking in Fish Bioconcentration Tests** M. McLachlan, Stockholm Univ, Dept of Applied Environmental Science; M. Adolfsson-Erici, G. Akerman, A. Jahnke, Stockholm Univ; P. Mayer, Aarhus Univ, National Environmental Research Institute; M. MacLeod, Stockholm Univ. The OECD 305 protocol for determining the bioconcentration factor (BCF) in fish is long, costly, and test animal intensive. Commonly an uptake phase of 28 days is followed by a depuration phase of up to 56 days, and a minimum of 40 fish are required. In addition, inter-laboratory precision of the results is not high. In an effort to improve quality while reducing the costs and test animal requirements, several innovative techniques were developed and tested including: -Passive dosing to maintain constant freely dissolved concentration of the target chemicals during the exposure phase -In-tissue passive sampling of the

target chemicals in the fish during the elimination phase -Internal benchmarking via simultaneous exposure of the fish to a chemical with known bioaccumulation behaviour. This led to a revised protocol shortened uptake and elimination phases (4/10 days), and a reduced number of fish (8). The revised protocol and the OECD 305 protocol were run for 10 chemicals using rainbow trout. Good agreement was found between the BCFs measured with the two methods. This supports the feasibility of reducing the cost and animal requirements of fish bioconcentration experiments. In addition to the methodological improvements provided by the passive dosing system, method precision was much improved thanks to the internal benchmarking. The revised protocol should be particularly useful in assessing whether chemicals lie above or below regulatory thresholds, as the internal benchmarking facilitates the determination of relative bioaccumulation behaviour.

**206 Use of Isolated Trout Hepatocytes to Predict Measured Hepatic Clearance and Whole-animal Bioconcentration Factors for Six Polycyclic Aromatic Hydrocarbons** K.A. Fay, P.N. Fitzsimmons, A.D. Hoffman, J.W. Nichols, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division. Hepatic metabolism is an important determinant of chemical bioaccumulation in fish. Consequently, measured in vitro hepatic metabolism may improve model predictions of bioaccumulation. In this study, fresh and cryopreserved trout hepatocytes were used to measure in vitro intrinsic clearance (CL<sub>in vitro</sub>) of six polycyclic aromatic hydrocarbons. These CL<sub>in vitro</sub> values were then extrapolated to the intact organ and compared to measured levels of clearance (CL<sub>H</sub>) by isolated perfused trout livers. To facilitate these comparisons, hepatocellularity scaling factors were determined for male and female trout. In addition, solid phase microextraction (SPME) methods were employed to determine unbound chemical concentrations in the isolated liver preparation. Generally, the hepatocytes performed well in predicting CL<sub>H</sub> rates exhibited by perfused livers. Although hepatocellularity values were higher for female trout than for males, predicted CL<sub>H</sub> values for the six compounds did not differ substantially. Finally, CL<sub>H</sub> values determined using both hepatocytes and isolated perfused livers were extrapolated to the whole animal and incorporated into a one-compartment bioconcentration model. Model-predicted bioconcentration factors (BCF) were then compared to measured values for strain- and temperature-matched trout. Preliminary results suggest that predicted BCF values are within the range of observed values.

**207 Predicting Field Variation of PCB Bioaccumulation in a Temperate Fish Population Under Steady State and Non-steady State Conditions** K. Drouillard, University of Windsor, Great Lakes Institute for Environmental Research. Much of the field of bioaccumulation has focussed on hazard assessment and establishing the potential for laboratory organisms to accumulate contaminants above regulatory criteria such as the BCF 5000 rule, to validate global predictions of food web bioaccumulation models or to measure trophic magnification factors at the food web scale. There have been fewer attempts to understand field variation of bioaccumulation metrics as it applies at the population scale in an indicator species and establish the magnitude and causes of field variability required for risk assessment purposes. More recently, non-steady state bioaccumulation dynamics have been recognized to occur in fish populations and yet field sampling designs rarely consider the implications of non-steady state bioaccumulation by fish or how this contributes to field variation in bioaccumulation metrics. This study used a modelling approach to contrast steady state and non-steady simulations of congener specific PCB bioaccumulation in a yellow perch population. The non-steady state bioaccumulation model, coupled with Monte Carlo analysis, considered uncertainty propagation among toxicokinetic parameters, seasonal changes in growth and weight loss and environmental heterogeneity in water and food contamination to simulate seasonal trends in PCB bioaccumulation, likelihood of achieving steady state for congeners of different hydrophobicity and to test whether model uncertainty would mask seasonal trends under realistic field sampling designs. Results of these simulations are contrasted against steady state model predictions to highlight where steady and non-steady state models predict different bioaccumulation patterns, for example congener ratios with age, to recommend sampling designs that could distinguish between steady and non-steady state bioaccumulation and to evaluate the implications of non-steady state bioaccumulation dynamics to field variability of chemical bioaccumulation.

**208 A Framework to Improve In Vivo Biotransformation Rate Constant Estimation** J. Arnot, ARC Arnot Research & Consulting Inc., Univ of Toronto Scarborough, Dept of Physical and Environmental Sciences; T.N. Brown, Helmholtz Centre for Environmental Research UFZ, Dept of Analytical Environmental Chemistry; E. Papa, Univ of Insubria, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology, Univ of Insubria, DBSF; P. Gramatica, Univ of Insubria, QSAR Res. Unit Environ. Chem. Ecotox./Dep. Structural & Functional Biology; W. Janzen, G. Schuurmann, Helmholtz Centre for Environmental Research UFZ; S. Dimitrov, Prof. Dr. Assen Zlatarov Univ, Dept of Comp. and Inform. Technologies, Univ of Zlatarov, Dept of Comp. and Inform. Technologies; O. Mekenyan, Prof. Dr. Assen Zlatarov Univ, Laboratory of Mathematical Chemistry; B. Meylan, P. Howard, Syracuse Research Corporation. Mass balance bioaccumulation models are required for bioaccumulation, exposure and risk assessment and to compare laboratory and field bioaccumulation metrics (e.g., BCF, BAF, BMF, TMF). The whole body primary metabolic biotransformation rate constant (kM) is a key bioaccumulation parameter required by the models and measured data are limited; therefore, reliable Quantitative Structure-Activity(Property) Relationships (QSA(P)Rs) are needed. A framework to improve kM estimation that maximizes the value of existing laboratory and field bioaccumulation information and provides intelligent testing strategies for future research is outlined and described. An empirical in vivo kM database for fish has been developed and various QSA(P)Rs are being developed and evaluated using different QSAR methods. The different QSA(P)Rs can be compared against the empirical data and against each other to identify potential sources of error in the existing database and merits and limitations of different QSA(P)R methods (e.g., for particular classes of chemicals and functional groups). This aspect of the framework is analogous in many ways to a laboratory "ring test" for chemical testing methods. A comparison of the different QSA(P)R predictions for thousands of organic chemicals provides the opportunity to identify chemicals with structural elements that are not currently well predicted for priority laboratory testing (in vivo and in vitro) in a defensible and efficient manner. New bioaccumulation testing data can then be obtained to expand the existing kM database and the domain of applicability in a second generation of QSA(P)Rs. This framework is expected to result in reduced animal testing, reduced uncertainty in kM estimation, reduced uncertainty in bioaccumulation, exposure and risk assessment, and improved data to compare laboratory and field bioaccumulation metrics.

**209 Connecting Uncertainty, Weight of Evidence, Data Consistency and Precaution for the Regulatory Assessment of Bioaccumulation** M. Bonnell, Environment Canada, New Substances Division, Environment Canada, Science and Risk Assessment Directorate. There are significant obstacles to developing a standard approach to making decisions on the bioaccumulation potential of a substance that can be adopted by all regulatory authorities. This is because an authority's approach will likely consider or weight evidence differently depending on regulatory context (e.g., the weighting of legal vs. non-legal metrics), the relevancy of different bioaccumulation metrics (e.g., in silico vs. in vitro vs. in vivo results) and how bioaccumulation is defined by a regulatory authority. Regulatory decision-making is also a "normative" process; that is, it incorporates considerations that are based on societal values. Therefore, probably the most important aspect of decision-making is that the process be transparent and reproducible to the degree feasible when using a normative process. This paper will discuss one possible approach to arrive at decisions on the bioaccumulation potential of substances from a regulatory perspective. In this approach, uncertainty is established based on the "strength of inference" a piece of information has to address the question posed by a null hypothesis (e.g., BCF or BAF is below the criterion value). The strength of inference is related to the quality and quantity of available data, and impacts both the weight given to individual pieces of information and the overall level of precaution applied in making the regulatory decision. The weighting of data can be performed using a qualitative or quantitative ranking method. As there can be many lines of evidence for bioaccumulation, the consistency between data or lack thereof should be examined. Lack of consistency adds to the overall level of uncertainty while greater consistency has the opposite impact. Precaution is linked to the degree of uncertainty when weighing evidence to test a hypothesis. In particular, a higher degree of precaution is used when the spread of uncertainty suggests that a false negative is possible in the above null hypothesis. A hypothetical chemical will be used to illustrate the above concepts using a qualitative weighting approach and data consistency matrix.

**210 Dispersants Evaluation, Use and Environmental Concentrations during the DWH Incident** A.D. Ahnell, BP Gulf Coast Restoration Organization, Science, Technology, Environment & Regulatory Affairs; G.M. Coelho, D.V. Aurand, Ecosystem Management & Associates, Inc.; J.S. Brown, Exponent Inc.; L. Bruce, BP, GCRO. This presentation will provide the context for evaluation and continued use of the dispersants applied to surface oil via aircraft and applied subsurface at the point of oil release. In combination, these data sets support the initial product selection and the large scale dispersant operations that provided an overall net environmental benefit during the spill response and were a key component of the response. The use of aerial dispersant applications as part of BP's initial spill response was one component of the pre-approved dispersant use plan. As application of Corexit products escalated in the initial phase of the response, BP and the USEPA tested additional dispersant products on the NCP list for efficacy and toxicity, but none performed significantly better and there were no significant differences in product toxicity to standard test species. Further support for continued use came from near-surface and sub-surface water sampling. Monitoring data by USCG SMART teams and BP contractors showed that dispersant applications to surface slicks created dispersed oil in the upper water column that rapidly diluted to levels that were not acutely toxic to standard test species in laboratory toxicity tests. Once subsea dispersant application was shown to be effective during initial tests, a monitoring plan commenced to collect data on the nature and extent of the subsurface dispersed oil. Particle size analysis and surface observations support dispersant effectiveness while dissolved oxygen concentrations showed only small decreases from enhanced biodegradation, and none exceeding the 2 mg/L lower limit set by USEPA. A large majority of the water samples collected and later analyzed by GC/MS showed oil and dispersant concentrations were orders of magnitude below acute toxicity effects thresholds for marine life. Data collected during the DWH incident provide significant insights into the net environmental benefit offered by surface and subsea dispersant applications. Further research should use the DWH incident data as context in selecting environmentally meaningful exposure regimes in when designing toxicology and biodegradation studies of physically and chemically dispersed oil. Due to the complexity of the topic and breadth of the information presented an extended time period is requested.

**211 Deepwater Horizon Long-Term Monitoring Study Shows Continuing Depletion of PAH in Oil and Sediment Samples from MC252-Impacted Areas** J.S. Brown, Exponent Inc.; L. Cook, Exponent; A. Ahnell, BP – GCRO. After the Deepwater Horizon (DWH) accident, certain Louisiana shorelines were impacted by MC252 oil. During the DWH response, the Long-Term Monitoring Program (LTM) was implemented by the Incident Command to study oiled shorelines in the absence of clean-up or remediation treatments to evaluate the natural weathering and biodegradation of MC252 in shoreline environments. The LTM study sites selected included key sensitive habitat types of the coastal region impacted by MC252 oil including: salt marsh, tidal channel marsh, phragmites marsh, mangrove, and shell berm. Additionally, un-oiled shoreline segments were selected as reference sites to characterize background hydrocarbons in marsh, mangrove, and shell berm habitats. Oil and sediment samples were collected at each site from the high tide, upper intertidal, and lower intertidal areas. Multiple sampling surveys were performed at each site to study changes over time. Samples were analyzed for polycyclic aromatic hydrocarbons (PAH), total and saturated petroleum hydrocarbons (TPH and SHC), and geochemical biomarkers (steranes and triterpanes). Sediment samples were also analyzed for total organic carbon (TOC) and grain size. Over a six month period, changes in PAH and SHC concentrations and chemical signatures in the oil and sediment samples were assessed to determine the processes acting on the petroleum hydrocarbons in each habitat type. Depletion of key chemical parameters was calculated for individual PAH, summed PAH, and summed alkanes using the conserved biomarker hopane, in comparison to fresh MC252 crude oil. MC252 impacted shoreline areas showed losses of PAH associated with dissolution, evaporation, biodegradation, and photo-oxidation. The majority of the depletion associated with dissolution, evaporation, and photo-oxidation likely occurred during the transport of the oil to the impacted areas. The earliest samples collected from these sites showed greater than 90% depletion for total PAH in all oil and sediment samples. Depletion of heavy PAHs (4-6 rings) and alkanes was not as rapid as total PAH and thus provided an extended range with which to observed trends with time. Continued depletion of heavy PAHs and alkanes was noted at several of the LTM sites, with further evidence of biodegradation.



**212 Identifying and Characterizing a Polar and Recalcitrant Fraction in Weathered Macondo 252 Well Oil** C. Aeppli, C.A. Carmichael, R.K. Nelson, C.M. Reddy, Woods Hole Oceanographic Institution, Dept of Marine Chemistry and Geochemistry. To investigate weathering of oil from the Deepwater Disaster one year later, we collected oil-soaked sand patties along the coasts of Mississippi, Alabama, and Florida as well as the Chandeleur Islands. These sand patties were easily found by searching for the orange-hue of the oil that was observed immediately after the spill. Analysis of the solvent extracts by gas chromatography with flame ionization detection (GC-FID), gas chromatography with mass spectrometry (GC-MS), and comprehensive two-dimensional gas chromatography (GCxGC) first confirmed by biomarker analysis that the oil was from the Macondo Well, that there were only traces of aromatic hydrocarbons, and that compounds with less than 17 carbons were no longer present. Furthermore, our analyses revealed that the oil-soaked sand patties generally contained less than 10% of solvent-extracted mass. Interestingly, only half of the extractable mass could be characterized by gas chromatography, presumably the other 50% of the material being too polar to be analyzed by GC. To investigate the polar nature of the extracts, we then analyzed the carbon, hydrogen, nitrogen, sulfur, and oxygen content. The whole extract from one sample contained 10% oxygen by mass while the original oil contained < 1% detectable oxygen. These results suggest that either abiotic or biotic oxygenation of the spilled oil caused such dramatic changes in the spilled. In addition, standard silica gel chromatography showed that the extract was evenly distributed between the saturated fraction and the polar fraction (eluting with hexane and dichloromethane/methanol, respectively). Together these results show that considerable weathering has occurred within one year since the spill. In order to identify the nature and products of such degradation processes, we plan to further characterize these polar fractions using infrared spectroscopy and liquid chromatography MS analysis. Nevertheless, this study has shown significant losses of hydrocarbons as well as changes consistent with oxygenation. Given the significant mass, and the so far poor understanding of the environmental fate of the described polar fraction, our findings imply that using commonly applied techniques solely based on GC amenable fractions to assess the environmental impact of the Deepwater Horizon incident may lead to a significant overestimation of natural oil attenuation.

**213 Laboratory Measurements of Dissolved and Droplet PAHs in MC252 Oil-Water-Dispersant Mixtures Inform Partitioning After Deepwater Horizon Oil Spill** P. Boehm, Exponent, Environmental & EcoScience Group, Exponent, Environmental Group, Exponent, Inc., Environmental & EcoScience sGroup; K. Murray, Exponent, Senior Scientist; D. Shea, North Carolina State Univ, Dept of Biology. The bioavailability of and risk from polycyclic aromatic hydrocarbons (PAH) from crude oil in water are dependent on whether they are dissolved or associated with droplets or particles. To adequately characterize the dissolved fraction of oil and its PAH constituents in a water sample and avoid the over-estimation of the dissolved PAH concentrations, any whole/droplet/particulate oil must be accounted for. There are two possible approaches to this problem: 1) the oil and water can be physically separated for analysis, or 2) the amount of the dissolved PAH can be calculated from the measured data from unfractionated samples using properties of PAH chemistry. To evaluate and understand the advantages and limitations of each approach, a series of laboratory experiments were conducted, and the results were compared to samples which were physically fractionated in the field after the Deepwater Horizon oil spill. Seawater and oil and seawater, oil, and dispersant mixtures were generated in the laboratory and then sampled. A whole, unfractionated sample was collected as were samples separated into particulate and dissolved fractions using two methods employed on ships during the field program. Alkanes and PAHs were measured in the dissolved and particulate fractions and corresponding unfractionated samples. Tested methods include the modified Payne filtration method (Payne et al. 1999) which uses a glass fiber filter to capture particles and direct analysis of the dissolved fraction in the filtrate and the Large Volume Sampler which utilizes a vortex separator and 0.45 µm filters for particulate analysis and sorption to a PUF cartridge to determine the dissolved concentrations. Results showed that an excellent mass balance in the experimental system (i.e., we could account for 100% of the material when comparing whole, unfractionated water samples to the sum of the fractions in the processed aliquot of the same sample). Reproducibility was good within each method though the composition of the fractions trapped by the filter (droplet) and passed through the filter ("dissolved") varied somewhat owing to differences in filter sizes. In no case

did the "dissolved fraction" resemble the water soluble fraction generated using standard WAF procedures. The analytical results were used to develop a method to calculate particulate concentrations from unfractionated water samples based on constant oil composition and minimally soluble individual oil components.

**214 Rapid Biodegradation of Aromatic and Saturated Hydrocarbons Associated with MC252 Oil in the Water Column and on the Shorelines of the Gulf of Mexico** R. Atlas, Univ of Louisville, Univ of Louisville, Dept of Biology; P. Boehm, Exponent, Environmental & EcoScience Group, Exponent, Environmental Group, Exponent, Inc., Environmental & EcoScience sGroup; L. Cook, Exponent, 1 Clock Tower Place. Physical and chemical dispersion of the MC252 oil released into the Gulf of Mexico between April 20 and July 15, 2010 resulted in detection of fine droplets at approximately 1100-1300 meters that generally moved in a southwesterly direction while larger droplets moved to the surface and formed a slick, with some of the oil moving northward and eastward to shorelines. Evidence for the biodegradation of the oil comes from detailed chemistry measurements using GC-MS analyses. These analyses showed a decreasing ratio of C17/pristane and C18/phytane as the oil moved away from the wellhead at 1100-1300 meters. Analyses based upon ratios of phenanthrenes and dibenzothiophenes and on ratios of high molecular weight aromatics and aliphatics to the conserved hopane confirmed extensive biodegradation of the aliphatic and polycyclic aromatic hydrocarbons in the deep sea. The extent of measured biodegradation was higher in the deep sea than in surface oil slicks where higher oil concentrations and lower surface area may have limited rates of biodegradation. Oil that spread out on shorelines also showed evidence of extensive biodegradation. The indigenous microorganisms were well-adapted to oil biodegradation. Microbial biodegradation of the oil removed many of the toxic components and reduced the overall impact of the oil released from the well.

**215 Microbial Gene Functions Enriched in the Deepwater Horizon Deep Sea Oil Plume** J. Zhou, Univ of Oklahoma. The Deepwater Horizon oil spill in the Gulf of Mexico is the deepest and largest offshore spill in US history and its impacts on marine ecosystems are largely unknown. Here, we used GeoChip-based high throughput metagenomic technology to show that the microbial community functional composition and structure were dramatically altered in a deep-sea oil plume resulting from the spill. GeoChip 4.0 contains more than 135,000 probes from 152,000 genes involved in C, N, S and P cycling, organic contaminant degradation, metal resistance, antibiotic resistance, stress responses, metal resistance, virulence, bacterial phage-mediated lysis, and soil beneficial microorganisms. A variety of metabolic genes involved in both aerobic and anaerobic hydrocarbon degradation were highly enriched in the plume compared to outside the plume, indicating a great potential for *intrinsic* bioremediation in the deep-sea. Various other microbial functional genes relevant to carbon, nitrogen, phosphorus, sulfur and iron cycling, stress responses, metal resistance, and bacteriophage replication were also enriched in the plume. Together, these results suggest that the indigenous marine microbial communities were capable of adaptation and succession in response to the oil spill and they could play a significant role in biodegradation of oil spills in deep-sea environments.

**216 Surface Dispersion and Biodegradation of the Macondo MC252 Crude Oil** P.S. Daling, SINTEF, Marine Environment Technology; O.G. Brakstad, SINTEF; F. Leirvik, SINTEF, Materials and Chemistry, Marine Environmental technology. At the Pensacola Seatac meeting in April, 2011, a weathering study was performed, including a weathering experiment in the SINTEF flume, where a high degree of natural dispersion were observed within 12 hours when the fresh released oil was exposed to simulated breaking wave sea conditions. Two series of follow-up experiments have recently been performed in the SINTEF flume weathering basin in order to get more detailed measurements of the droplet size distribution generated from the surfacing oil both due to natural dispersion under breaking wave conditions, and chemically enhanced dispersions by using low dosages of Corexit 9500. A LISST-100X Laser Particle Sizer, a particle visual microscope (PVM) and a high resolution camera were used for documenting the droplet size distribution. The oil loading used in the flume experiments were reflecting a "realistic" dispersed oil concentrations (20 – 100 ppm) that can be obtained in the upper meter of the surface water column in the field short time after a re-dispersion of the surface oil slick. Physically and chemically dispersed

flume experiment samples were collected for studies of dispersed oil biodegradation. Samples were distributed into closed vials and incubated for a period up to 2 months at the relevant GoM sea surface temperatures (30-32 °C) with continuous agitation. The samples were removed for analyses at pre-defined intervals. Some samples were used as "abiotic" controls by addition of biocide (HgCl<sub>2</sub>). The biodegradability of various oil components (alkanes, aromatics, iso-prenoides) were determined by comparing the result from biotic versus the abiotic samples using GC-FID and GC-MS. In addition, the biodegradability of 10-20 µm thick oil films (also representative for "metallic" oil films) immobilized on hydrophobic absorbents were examined in closed vials with normal pre-conditioned (30-32°C) seawater and with a biodegradation period of up to 28 days. Fabrics with immobilised oil in sterile seawater were used as controls (seawater poisoned with HgCl<sub>2</sub>). Fabrics were removed for analyses at pre-defined intervals for oil extraction and analyses by GC-FID and GC-MS. Biodegradation was determined by comparison of results from biotic samples and abiotic controls and also compared to previous similar experiment with other crude oils under other (North Sea) environmental conditions. The results from this surface dispersion and biodegradation study will be presented.

#### 217 Natural Oil and Gas Seeps and Oil Slicks in the Mississippi Canyon Protraction Area: Origins and Historical Baseline before the DWH Incident

**P.D. Carragher**, BP Gulf Coast Restoration Organization, Science, Technology, Environmental and Regulatory Affairs; **A. Kornacki**, Weatherford, BP GCRO, 1315 West Brooklake; **C. Scherschel**, BP, BP, 1315 West Brooklake; **R. Frost**, BP, Upstream Technology, 1315 West Brooklake. Oil and gas seepage is a pervasive occurrence in the Gulf of Mexico that has existed for many millions of years. This paper will trace the transport of hydrocarbons from deep in the earth to the sediment water interface, and then to their ultimate fate as a natural surface slick is weathered. Seep data obtained from before the DWH oil spill will be reviewed to provide a view of the baseline or naturally existing system. With the advent of deepwater exploration, data exists that enables us to establish the geological origin of the petroleum in natural seeps that forms at depths of greater than 15,000ft below the surface; track the oil and gas migration to the seabed, often along the sides of salt domes; map and visualize the expression of the seeps in the shallow sediments and at the sea floor; and, in the case of gas seeps in particular, document their rise through the water column. Naturally occurring surface oil slicks are detectable by satellite and other remote sensing capabilities. Carbonate hardgrounds of biological origin are formed by, and sustained for periods of time by seeping hydrocarbons. Other fauna anchor on these hardgrounds and build complex and diverse deepwater communities. Sediments in the vicinity of natural seeps contain complex mixtures that may include some or all of the following components; – fresh oil, biodegraded oil, hydrocarbon gases of thermal and/or microbial origin, and recent organic matter. Quantitative methods that separate the relative contribution of these components have been applied to more than a hundred deep water sediment samples that pre-date the DWH incident. Preliminary interpretation of these data will be presented. Natural seepage may be continuous or occur in a series of geyser-like eruptions, and over time this episodic process creates a variety of seabed craters and other features. Preliminary results of an AUV survey covering an area of natural seeps will be presented to illustrate these conditions. Naturally occurring surface oil slicks are present on the surface of the sea in the vicinity of the Mississippi Canyon Protraction area. Preliminary results from a satellite based study documenting the location, size and area of these natural slicks will be presented, along with examples of the tie to natural seepage on the seafloor and the deep subsurface origin. Supporting material will be presented in the accompanying posters.

#### 218 Introduction of a Quantitative Label-free, Non-gel Based Proteomic Method for Use in Aquatic Ecotoxicological Study of Small Fish Species

**K. Ralston-Hooper**, Visiting Dupont Research Scientist at Duke Univ, Nicholas School of the Environment; **R. Hoke**, DuPont, Haskell Global Centers; **D.L. Villeneuve**, USEPA, Mid-Continent Ecology Division; **G.T. Ankley**, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; **M. Turner**, **E. Soderblum**, **A. Moseley**, Proteomics Core Facility, Institute for Genome Science & Policy, Depts of Cell Biology, Medicine, and Neurobiology, Duke Univ Medical Center; **L. Ferguson**, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Nicholas School of the Environment, Pratt School of Engineering, Dept of Civil & Environmental Engineering.

Two-dimensional electrophoresis remains one of the most exploited techniques for protein separation but significant limitations are associated with this gel-based method. This particular technique remains cumbersome, time consuming, and results in the identification of a small number of proteins (30-50% of proteome). A non-gel, label-free, high throughput technique could facilitate the realization of the full potential of proteomics in ecotoxicology. Our goal is to apply an open, unbiased, label free, non-gel based proteomics technique to determine differentially expressed proteins in the fathead minnows (FHM), *Pimephales promelas*. Initial research has demonstrated that proteins were successfully extracted, digested, and analyzed using ultra pressure liquid chromatography coupled with mass spectrometry followed by spectral search against NCBI Actinopterygii (ray-finned fishes) database. The number of unique proteins in brain, gonad, and liver samples were 159, 155, and 167, respectively. Many of these proteins were associated with metabolic and cellular processes. This method was then applied to examine differential proteomic profiles of FHM exposed to the potent anti-estrogen, Fadrozole (FAD), at concentrations of 0, 0.04, and 1 µg/L. Liver tissues of exposed organisms were extracted, digested, and analyzed. These differentially expressed proteins provide valuable insight into how endocrine disrupting compounds (EDC) perturb the CYP pathway resulting in adverse effects. The overall goal of this project is to serve as a "proof-of-concept" evaluation of ecotoxicologically-relevant tissues using a label-free, non-gel based proteomics method. This high throughput proteomics technique coupled with other "omic" technologies will provide a systems biology approach to examine mechanism of action of environmental contaminants.

#### 219 Leveraging Semantic Integration of Web and Experimental Resources in Interpreting Toxicology Data

**M.M. Hindle**, **A. Riazanov**, Univ of New Brunswick, Dept of Computer Science & Applied Statistics; **C.J. Martyniuk**, Univ of New Brunswick, Canadian Rivers Institute and Dept of Biology; **C.J. Baker**, Univ of New Brunswick. In order to interpret experimental omics data, toxicologists are faced with a bewildering array of disconnected bioinformatics databases and tools. For example, tools for microarray analysis, gene annotation, functional gene set enrichment, and network analysis. Drawing together web tools and resources is frequently an unnecessary and frustrating technical exercise in identifying links across database records and the connecting input and output formats of tools. Interpreting experimental omics data in the context of the current available knowledge and methodologies from a single query platform with explicit semantics would be a valuable asset for ecotoxicology in the analysis of their DNA, transcriptomics, proteomic, and metabolomic experimental data. We have created 30+ SADI semantic web services, resources and tools pertinent to the interpretation of omics toxicological data. These services expose a wide range of algorithms, domains and databases: sequence alignment and protein domain finding tools (e.g., BLAST, HMMER3, and InterProScan), databases containing experimentally validated protein functions (e.g., ZFIN and MGI), and central repositories of sequence and microarray data (e.g., GEO and NCBI-RefSeq). All these services can be leveraged through queries submitted to a SHARE web client. This paradigm provides a single access-point on the web for a toxicologist to submit semantically rich queries, which are answered using appropriate databases and tools. This frees the toxicologist from learning unnecessary details concerning tool interfaces, and the semantic idiosyncrasies of databases. It also has the potential to reveal otherwise hidden links between experimental data and is readily scalable to high throughput omics data. We present a series of exemplar queries for toxicology, which facilitate the interpretation of transcriptomics data in the context of public knowledge and current tools. These queries include common tasks, specific to a user's experimental data set, such as the annotation of probes with the gene ontology for a custom fish microarray experiment. The example queries also address broader open-world queries, such as the impact of a toxicant on the transcriptome, which is based on evidence in published data. These queries demonstrate how a toxicologist can use semantic web services to better understand their data using the most recent public knowledge, and view their results in the context of other published experimental results.

#### 220 Metabolomic Insights into Thyroid Hormone Action and Disruption

**C. Helbing**, Univ of Victoria, Dept of Biochemistry & Microbiology; **M. Lesperance**, **L. Lu**, Univ of Victoria; **J. Han**, **C. Borchers**, UVic-Genome BC Proteomics Centre; **R. Ng**, **M. Takhar**, Univ of British Columbia; **N. Veldhoen**, Univ of Victoria; **R.C. Skirrow**, **G. van Aggelen**, Pacific Environmental Sciences Centre, Environment Canada. Frog tadpoles require

appropriate thyroid hormone signalling to undergo metamorphosis into a froglet. Exogenous administration of thyroid hormones to young tadpoles induces a precocious metamorphosis and substances that alter thyroid hormone action will impact this postembryonic developmental process. Very little is known about serum-associated metabolites within the tadpole that change in response to thyroid hormone and identification of key metabolites could serve as effective biomarkers for thyroid hormone disruption. We injected premetamorphic *Rana catesbeiana* (American bullfrog) tadpoles with 10 nM 3,5,3'-triiodothyronine in the presence and absence of exposure to nanosilver and analyzed the serum metabolites after 2 days post-injection. Serum metabolites were liquid/liquid extracted into organic and aqueous phases following protein precipitation and analysed using direct infusion-Fourier transform ion cyclotron resonance mass spectrometry (DI-FTICR MS). The same samples were also examined with ultra performance liquid chromatography (UPLC)–MS using BEH C-8 and HSS-T3 columns. Metabolites were ionized by electrospray and detected in both positive-ion and negative-ion modes. We identified over 10,000 metabolite features with both techniques. Metabolites identified in lipid metabolism, bile acid synthesis, and nucleotide metabolism were found to be differentially present using nonparametric and correspondence analyses, suggesting that these pathways are important targets for thyroid hormone action. The utility of a serum metabolite approach for the detection of thyroid hormone disruption will be discussed.

**221 Assessment of the Relative Contribution of Wastewater Treatment Plant Effluents to the Adverse Impacts on Fish Health – A Genomic Approach** D. Martinovic-Weigelt, Univ of St. Thomas, Dept of Biology, USEPA, Mid-Continent Ecology Division, Mid-Continent Ecology Division, Univ of St. Thomas, Biology; A. Mehinto, Univ of Florida; J. Miller, St. Cloud State Univ, Biosciences, St. Cloud State Univ; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology; H. Schoenfuss, St. Cloud State Univ, Aquatic Toxicology Laboratory, St. Cloud State Univ, Dept of Biological Sciences MS-273 The effects of wastewater treatment plant (WWTP) effluents on fish were studied at three locations, selected to represent a variety of treatment technologies, population sizes and geographic distributions. At each location, a mobile exposure laboratory trailer was set up to conduct 48-hr exposures of male fathead minnows to the wastewater effluent, and upstream and downstream receiving waters. Following the exposures we analyzed gene expression in the livers using a 15,000 gene microarray developed specifically for fathead minnows. Gene expression in fish was altered when they were exposed to WWTP effluents or to the surface water collected either upstream or downstream of WWTPs. One of the prominent observations made in the microarray study was that all the effluents had an effect on genes that regulate iron metabolism and immune responses in fish. Overall, WWTP effluents had an effect on gene expression that was more similar to downstream sites than to upstream sites, suggesting a detectable effluent signature at all of the downstream sites. These findings suggest that WWTPs are an important contributor to aquatic pollution, and that they have potential to adversely impact multiple physiological pathways important for an organism's fitness (immune system functioning, reproductive success and aerobic metabolism), which is consistent with the complex composition of WWTP effluents. We are currently integrating the results of this study with longer term exposures (4, 8, 14 d), conducted at the same locations, in an attempt to determine if the observed effects on gene expression can be related to the apical endpoint effects (e.g., histopathology, reproductive health, plasma vitellogenin, and secondary sex characteristics).

**222 Histaminergic Signaling in the Central Nervous System of *Daphnia* and a Role for it in the Control of Phototactic Behavior** M. McCool, The Univ of Louisiana at Monroe, Dept of Toxicology; K. Baer, The Univ of Louisiana at Monroe, College of Pharmacy; A. Christie, Mount Desert Island Biological Laboratory. *Daphnia magna* and *Daphnia pulex* are well-established model organisms in the fields of ecotoxicology and toxicogenomics. Among the many assays used for determining the effects of environmental and anthropogenic stressors on these animals is monitoring for changes in their phototactic behavior. In most arthropods, histamine has been shown to play a key role in the visual system. Currently, nothing is known about histaminergic signaling in either *D. magna* or *D. pulex*. Here, a combination of immunohistochemistry and genome mining was used to identify and characterize the histaminergic systems in these daphnids. In addition, a behavioral assay was used to assess the role of histamine in

their phototactic response to ultraviolet (UV) light exposure. An extensive network of histaminergic somata, axons and neuropil was identified via immunohistochemistry within the central nervous system of both daphnids, including labeling of putative photoreceptors in the compound eye and projections from these cells to the brain. Mining of the *D. pulex* genome using known *Drosophila melanogaster* proteins identified a putative ortholog of histidine decarboxylase (the rate-limiting biosynthetic enzyme for histamine), as well as two putative histamine-gated chloride channels (hclA and hclB orthologs). Exposure of *D. magna* to cimetidine, an H<sub>2</sub> receptor antagonist known to block both hclA and hclB in *D. melanogaster*, inhibited their negative phototactic response to UV exposure in a reversible, time-dependent manner. Taken collectively, our results show that an extensive histaminergic system is present in *Daphnia* species, including the visual system, and that this amine is involved in the control of phototaxis in these animals.

**223 Metabolite Profiling and Receptor Binding Assay Provide Direct Physiological Evidence of Androgen Receptor Antagonism by Bisphenol A in Fish** D. Ekman, USEPA, National Exposure Research Laboratory; P. Hartig, M. Cardon, T. Collette, E. Durhan, E. Gray, K. Jensen, M. Kahl, Q. Teng, D. Villeneuve, G. Ankley, USEPA. Widespread environmental contamination by bisphenol A (BPA) has created the need to fully define the mode(s) of action (MOA) of this pollutant in order to properly assess risk. Long recognized as an estrogen receptor (ER) agonist, BPA has recently also been suggested as having androgen receptor (AR) antagonistic properties. However, direct physiological evidence of this activity has not yet been established. In order to address this deficiency, we employed a metabolomic approach using in vivo exposures of fathead minnows (FHM) to BPA either alone or in co-exposure with 17b-trenbolone (TB), a strong AR agonist. Changes in liver metabolite profiles in female FHM in response to these exposures were determined using high resolution <sup>1</sup>H-NMR spectroscopy and multivariate and univariate statistics. Using this approach, we observed direct evidence of the ability of BPA to mitigate the impact of TB exposure, consistent with an anti-androgenic mode of action. In addition, urinary metabolite profiles in male FHM exposed to BPA revealed signatures similar to those produced by exposure to the recognized antiandrogens vinclozolin and cyproterone acetate. Finally, an in vitro transcriptional activation assay was used to confirm the AR antagonistic activity of BPA to the FHM AR. The results of these varied analyses (both in vivo and in vitro) provide several strong lines of evidence for ascribing an anti-androgenic MOA to BPA in addition to the established ER agonism.

**224 Effects of Bisphenol A on the Ovarian Transcriptome of Two Small Fish Species** D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; N. Garcia-Reyero, Jackson State Univ, Dept of Chemistry; L. Escalon, US Army Engineer Research and Development Center, Environmental Laboratory; K.M. Jensen, USEPA, Mid-Continent Ecology Division; J. Cavallin, USEPA; E. Makynen, E. Durhan, M. Kahl, L. Thomas, USEPA, Mid-Continent Ecology Division; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division. Bisphenol A (BPA) is a high production volume chemical widely used in the manufacture of polycarbonate plastics, epoxy resins, and many other commercial products. BPA has long been characterized as a xenoestrogen, and recent work suggests potential additional modes of endocrine action. To improve our understanding of the potency and diversity of BPA's effects at the molecular level, effects of the chemical on ovarian transcript profiles as well as targeted endpoints with endocrine/reproductive relevance were examined in two fish species, fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*), exposed in parallel using matched experimental designs. Four days of waterborne exposure to 10 µg BPA/L caused significant vitellogenin induction in both species. However, zebrafish were less sensitive to effects on hepatic gene expression and steroid production than fathead minnow and the magnitude of vitellogenin induction was more modest. The concentration-response at the ovarian transcriptome level was non-monotonic and violated assumptions that underlie proposed methods for estimating hazard thresholds from transcriptomic results. However, the non-monotonic profile was consistent among species and there were nominal similarities in the functions associated with the differentially expressed genes, suggesting potential activation of common pathway perturbation motifs in both species. Overall, the results



provide an effective case study for considering the potential application of ecotoxicogenomics to ecological risk assessments and provide novel comparative data regarding effects of BPA in fish. The contents of this abstract neither constitute nor reflect official USEPA policy.

**225 eMICE 2011 – An Environmental Metabolomics Intercomparison Exercise** D. Bearden, National Institute of Standards and Technology, Analytical Chemistry Division, National Institute of Standards and Technology; M.R. Viant, J. Byrne, NERC Biomolecular Analysis Facility – Birmingham Node; A. Boroujerdi, T. Schock, National Institute of Standards and Technology, Analytical Chemistry Division. Previous results of an international intercomparison exercise for NMR-based environmental metabolomics showed that NMR metabolome analysis yields robust results with consistent trends in metabolite-based biomarker identification among laboratories. This type of demonstrated comparability strengthens confidence in the concept as the technique is considered for regulatory environmental studies. Based on the lessons learned from the first effort, a second exercise was developed, and updated data collection and processing protocols were used, together with a considerably larger number of international participants, to further investigate the comparability and suitability of NMR-based metabolomics data for environmental research, assessment and regulatory roles. The exercise design emphasizes the steps that occur after sample collection and initial sample preparation. The samples used for the exercise were a set of simplified artificial metabolite mixtures and a set of fish muscle extracts from a marine aquaculture feeding study. The data collection protocol was extended to include 2-dimensional (2D) JRES spectra and additional measurements involving temperature calibration and signal-to-noise ratios. In the end, 13 labs contributed 16 data sets from instruments operating at 4 different magnetic field strengths. Factors to be evaluated in the exercise include the final stages of sample preparation, NMR data collection at multiple NMR-field strengths, quantitative evaluation and multivariate data analysis (principal component analysis). Several data quality evaluation protocols for multivariate data sets have been utilized to assess participants' success with the exercise. This presentation will provide an analysis of the second intercomparison results.

**226 Application of EPA Guidelines in a Bioavailability-based Assessment of Ambient Water Quality Criteria for Zinc** D.K. DeForest, Windward Environmental LLC; E.J. Van Genderen, International Zinc Association, Assistant Manager, Environment. The United States Environmental Protection Agency's (EPA's) current ambient water quality criteria for zinc in fresh water are hardness-based and were last updated in 1995. Since then, two significant advancements have been made regarding the assessment of zinc toxicity to aquatic organisms. First, the acute and chronic ecotoxicity databases have been complemented by several new studies and taxonomic groups, thereby satisfying the EPA's minimum phylogenetic diversity requirements for chronic zinc toxicity (chronic criteria were historically derived using an acute-to-chronic ratio). Second, several acute and chronic biotic ligand models (BLMs) for zinc have been developed and validated for fish, invertebrates, and algae. Considered together, the objective of this effort was to develop a zinc BLM that could efficiently predict both acute and chronic toxicity from published studies that evaluated a wide range of water chemistries (e.g., dissolved organic carbon, pH, hardness). Although several of the published zinc BLMs demonstrated good predictions for the available datasets, a unified model was objectively generated by averaging the biotic ligand binding constants for zinc ( $Zn^{2+}$ ) and competing cations ( $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ ,  $H^+$ ) from all studies. Moreover, toxicity predictions were further improved by optimizing the biotic ligand binding constant for the  $ZnOH^+$  species. Only the critical accumulation parameter for each species/endpoint was changed during the auto-validation of each BLM (published or unified). Following selection of the optimized model, normalized species sensitivity distributions (using geometric mean critical accumulation concentrations for each species/exposure type) were constructed for eight water types for the estimation of 5<sup>th</sup> percentile effect concentrations (analogous to EPA's final acute values and final chronic values). A comparison of results from this assessment and historical criterion derivation practices will be discussed.

**227 A Retrospective Analysis of Manganese in Lake Trout (*Salvelinus namaycush*) Otoliths: Links to Reproductive Failure** L.C. Carroll, Univ of Manitoba, Dept of Environment and Geography, Fisheries and Oceans Canada, Freshwater Institute, Freshwater Institute, Univ of Manitoba,

Environment and Geography; N. Thebeau, Ministry of Natural Resources, Red Lake District; N. Halden, Univ of Manitoba, Dept of Geological Sciences; M. Hanson, Univ of Manitoba, Dept of Environment and Geography, Univ of Manitoba, Faculty of Environment; V. Palace, Dept of Fisheries & Oceans Canada, Dept of Fisheries and Oceans. Microchemical analysis of an increasing number of trace elements in fish otoliths has become an important tool to determine environmental exposure. Over the last decade a decline in abundance of the lake trout (*Salvelinus namaycush*) population of Red Lake, Ontario has been documented, coincident with apparent recruitment failure. Since 2001 spawning assessments, surveys, and bioassays focussed on the trout's primary breeding shoals in Red Lake's Pipestone Bay have been conducted to attempt to discover the causal agent for the ongoing recruitment failure. The Red Lake area has a history of gold mining so that metals associated with this industry warrant attention. Manganese (Mn) is associated with mining, and previous water quality data from Red Lake show elevated concentrations. Mortality was also associated with Mn in an acute toxicity study performed by local authorities. An analysis of Mn in archived lake trout otoliths (1963-2008) was performed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Manganese concentrations in otolith from lake trout captured from Red Lake and from two reference sites (Trout Lake and Confederation Lake) were compared to determine if temporal variations supported a role for Mn in recruitment failure. Over a ten year period Mn concentrations in otoliths obtained from the three lakes were: Pipestone bay: 0.00 – 6.61  $\mu g/g$ ; Trout Lake: 0.00 – 4.24  $\mu g/g$ ; Confederation Lake: 0.00 – 10.19  $\mu g/g$ . There were significant differences ( $P < 0.001$ ) in yearly Mn concentration in all three lakes. It appears that Mn is incorporated into otoliths at low concentrations. Mn concentrations were highest in otoliths from Confederation Lake compared to the other two lakes. Even though Mn concentrations were low, peaks associated with certain time periods could be discerned. There was no mining activity directly on Red Lake or Trout Lake, however, elevated Mn concentrations were measured in otolith sections corresponding to the period between 1980 and 1990. These results do not allow Mn to be ruled out as a causal agent of lake trout recruitment failure in Red Lake.

**228 Application of a Multi-metal BLM to Predict Toxicity in Metal Contaminated Sediments from the TSMD. 1 – Background, Sampling, and Analysis Methods** W. Brumbaugh, US Geological Survey, Columbia Environmental Research Center; C.G. Ingersoll, USGS, Columbia Environmental Research Center; J.M. Besser, US Geological Survey, Columbia Environmental Research Center; N.E. Kemble, US Geological Survey, Columbia Environmental Research Center, USGS – Biological Resources Division, Columbia Environmental Research Center; D. MacDonald, MacDonald Environmental Sciences Ltd.; R. Santore, HDR|HydroQual; A. Ryan, HDR|HydroQual; P. Paquin, HDR|HydroQual. The Tri-State Mining District (TSMD) is an historic lead and zinc mining area that includes portions of Kansas, Missouri, and Oklahoma, USA. Historic mining activities in the area have resulted in contamination of surface water, groundwater, sediments, and flood plain soils by lead, zinc, and other heavy metals. As part of an advanced screening level ecological risk assessment being conducted by the US Environmental Protection Agency (USEPA), we conducted comprehensive chemical analyses and whole-sediment toxicity assessments for stream sediments collected from 76 locations within the TSMD. Sediments were analyzed for grain size, fraction of organic carbon (foc), acid-volatile sulfide (AVS), simultaneously-extracted metals (SEM), total recoverable metals, polycyclic aromatic hydrocarbons, and organochlorine compounds. Pore water, sampled by centrifugation/filtration just before onset of 28-d toxicity testing with the amphipod *Hyalella azteca* and by in situ dialysis (peepers) on days 7 and 28 during testing, was analyzed for dissolved organic carbon, major cations and anions, and trace metals. For this project, availability of high quality sediment pore water data which includes a comprehensive list of metals, as well as factors known to affect metal bio-availability allowed us to perform Biotic Ligand Model (BLM) calculations on sediment pore waters. To perform these calculations, the existing BLM was modified to allow simultaneous simulations for individual metals such as Cd, Cu, Ni, Pb, and Zn. This combined simulation allowed the model to consider chemical interactions between metals, including chemical competition for binding sites on organic and inorganic ligands. This presentation serves as an introduction to the companion presentation that describes the results of the BLM calculations.

**229 Application of a Multi-Metal BLM to Predict Toxicity in Metal Contaminated Sediments from the TSMD: Pt. 2 – BLM Modeling** R. Santore, A.C. Ryan, P. Paquin, HDR|HydroQual; C.G. Ingersoll, USGS, Columbia Environmental Research Center; W. Brumbaugh, J.M. Besser, US Geological Survey, Columbia Environmental Research Center; N.E. Kemble, US Geological Survey, Columbia Environmental Research Center; D. MacDonald, MacDonald Environmental Sciences Ltd. A variety of methods are available for estimating potential risks associated with metals in sediments, including empirically based guidelines (e.g., TECs and PECs) for total concentrations in bulk sediments, and comparison of mechanistically based guides (e.g., SEM and AVS) with or without consideration of sediment organic matter. When pore-water metal concentrations and general water chemistry is available, comparisons against water quality criteria are possible according to equilibrium partitioning theory. For this project, 76 sediment samples collected across a gradient of metal contamination from the Tristate Mining District in Missouri, Kansas and Oklahoma were evaluated using 28-d whole-sediment toxicity tests with the amphipod *Hyalella*. Sediments were characterized with high quality sediment pore-water data and whole-sediment data including a comprehensive list of metals, as well as factors known to affect metal bioavailability (Brumbaugh et al.), which allowed us to perform Biotic Ligand Model (BLM) calculations on sediment pore waters. To perform these calculations, the existing BLM was modified to allow simultaneous simulations for metals including Cd, Cu, Ni, Pb, and Zn. This combined simulation allowed the model to consider chemical interactions between metals, including chemical competition for binding sites on organic and inorganic ligands. Interactions between metals on biotic ligand sites were also incorporated. For these samples, BLM results indicated that observed toxicity was most likely to be associated with Cd, Pb, and Zn. Since these metals are all associated with inhibition of calcium ion uptake, the assumption in the multiple-metal BLM is that their interactions occur on the same biotic ligand site, and their effects are additive. In general, the BLM running all metals simultaneously and considering Cd, Pb, and Zn effects in a dose-additive way produced a better comparison with observed toxicity than the BLM with any one metal alone. Performance of the BLM for identifying which samples were toxic was compared with other sediment screening methods such as the use of TECs, PECs, and SEM-AVS and was generally more accurate in differentiating sediments where toxicity to *Hyalella azteca* was observed.

**230 Metal Classification Using a Unit World Model: Assessing Removal Rates from the Water Column and Remobilization from Sediment with Ticket-UWM** K.J. Rader, Mutch Associates, LLC, Manhattan College, Civil and Environmental Engineering; R.F. Carbonaro, Manhattan College, Civil and Environmental Engineering, Manhattan College, Dept of Civil and Environmental Engineering; K.J. Farley, Manhattan College, Civil and Environmental Engineering, Manhattan College, Civil & Environmental Engineering. In the European Union, regulations pertaining to Classification, Labeling, and Packaging (CLP) of chemical substances/mixtures follow the United Nations Globally Harmonized System (UN GHS). Recently, an update to the classification system for metals and sparingly soluble metal compounds (SSMC) has been proposed that includes a new paradigm for long-term aquatic hazard assessment. Included in the update is a provision for demonstrating removal from the water column to assess the “persistence” of metals. In analogy to organic chemicals, removal of the “persistent” classification for metals requires greater than 70% removal within 28 days. However, unlike organic chemicals where removal from the water column can occur via degradation, metal removal occurs through sorption/precipitation and sedimentation processes which transfer metal to the sediment. Therefore, removal of the “persistent” classification for metals requires one to demonstrate not only rapid removal from the water column, but also limited remobilization potential from sediment. A unit world model for metals in lakes, TICKET-UWM, has been developed that builds on screening-level hazard assessment calculations for organic chemicals. This model considers key processes affecting metal transport, fate, and toxicity—several of which are unique to metals. These include complexation by aqueous ligands such as dissolved organic carbon (DOC), adsorption to particulate phases such as particulate organic carbon (POC) and iron/manganese oxides, binding to biological receptors (biotic ligands), dissolution kinetics of metal powders, cycling of organic matter and sulfide production in lakes, and transport of dissolved metals and solids between the water column and sediment. To assess its use as a screening-level aquatic hazard assessment

tool, the TICKET-UWM was used to simulate removal rates of several metals including Cu, Ni, Zn, and Pb from the water column of a generalized lake system and from actual lakes. In general, both model-predicted and observed removals exceeded 70% in 28 days. The TICKET-UWM was able to reproduce observed water column copper dynamics reasonably well. Remobilization assessments suggest that feedback from the sediment for single dose events has a generally limited impact on water column concentrations. Metal precipitation with sulfide in sediment is important for sequestering metals and preventing loss to the water column via diffusive fluxes.

**231 The Use of Biotic Ligand Models in Environmental Risk Assessments: An European Perspective** P. Van Sprang, ARCHE. Recently EU risk assessment reports on copper, nickel and zinc has been finalized and adopted. In the environmental effects assessment chapter chronic toxicity data for different trophic levels have been compiled and thoroughly screened for the derivation of the species sensitivity distribution (SSD). Safe threshold concentrations are subsequently calculated as the lower 5<sup>th</sup> percentile of the SSD. In addition, for all metals mentioned bioavailability corrections using chronic Biotic Ligand Models (BLM) were used to normalize the chronic effects data of the SSDs for different selected EU water conditions at both the local and regional scale and compared with monitoring data of dissolved metal. This presentation aims at providing an overview of the approaches used in Europe for a tiered bioavailability correction for the metals Cu, Ni and Zn. The differences in strategy used between the EU and US for the development and validation of BLM will be highlighted. Furthermore the presentation will focus on the predictability and applicability of the BLMs towards different surface water conditions and for different species. Finally, the incorporation of the bioavailability correction into the EU regulatory context will be presented.

**232 The Use of Multi-Linear Regression to Derive Site-Specific Water Quality Criteria for Metals: A Complementary Approach to the Biotic Ligand Model** K.V. Brix, Univ of Miami, RSMAS, Marine Biology and Fisheries; D.K. DeForest, Windward Environmental LLC; L.M. Tear, Windward Environmental; A.C. Ryan, HDR|HydroQual; M. Grosell, Univ of Miami, RSMAS; W.J. Adams, Rio Tinto, Product Stewardship. Over the past 15 years there has been a concerted effort to develop Biotic Ligand Models (BLMs) for the derivation of site-specific US-based Water Quality Criteria (WQC) or European Environmental Quality Standards (EQS) for metals. BLMs are now available for a range of metals and are widely applied in ecological risk assessments. In Europe, both regional and national water quality criteria are under development that will incorporate BLMs, and in North America the USEPA has promulgated a BLM-based WQC for Cu in freshwater. Despite its availability, to date, no state in the US has adopted this new criterion into its numeric standards. There are likely a number of reasons for this, but the perception that the BLM is too complicated is frequently cited as a reason for lack of adoption at the state level and the USEPA has postponed development of BLM-based WQC for other metals as a result. To address this issue, we developed a complementary approach to the BLM, which begins by using the BLM framework to identify key water quality parameters influencing metal toxicity. These key parameters are then used to develop simple multi-linear regression (MLR) models that predict metal toxicity on a species-specific basis. Regressions from species-specific MLRs are then pooled in a manner analogous to the pooling of slopes used in previous US-based hardness-dependent WQC. The resulting final criteria equations are equivalent in form with hardness-dependent WQC equations, but with one or two additional terms (typically dissolved organic carbon and pH). Comparison of MLR-based versus BLM-based WQC for Cu, Ni, Pb, and Zn indicates MLR performance is comparable to the BLM. We suggest that this MLR-based approach, which includes a mechanistic foundation like the BLM, but requires less input data and has an output that is similar to the widely acceptable hardness-dependent WQC, may be more amenable to adoption at the state level.

**233 Transformation/Dissolution Studies on Tungsten Metal and Compounds, with Speciation of the T/D Solutions** J.M. Skeaff, D.J. Hardy, CANMET-MMSL, Natural Resources Canada. Speciation is held to be a key factor in controlling the ecotoxicity of metals in solution. We have applied the UN Transformation/Dissolution Protocol (T/DP) for Metals and Sparingly Soluble Metal Compounds to examine the T/D characteristics of W metal and several W compounds at pH 6 and 8.5 at loadings of 1, 10 and 100 mg/L: sodium tungstate, Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O; yellow tungsten



trioxide, WO<sub>3</sub>; three samples of tungsten carbides, WC; tungsten metal, W; ammonium paratungstate (APT), (NH<sub>4</sub>)<sub>10</sub>(H<sub>2</sub>W<sub>12</sub>O<sub>42</sub>).4H<sub>2</sub>O; ammonium metatungstate (AMT), (NH<sub>4</sub>)<sub>6</sub>(H<sub>2</sub>W<sub>12</sub>O<sub>40</sub>).6H<sub>2</sub>O, and blue tungsten oxide, WO<sub>x</sub> (x assumed to be 2.9). All presented a degree of reactivity with the aqueous media in terms of total dissolved W and the WO<sub>4</sub><sup>2-</sup> anion. The generally good agreement between the concentrations of total dissolved W and the WO<sub>4</sub><sup>2-</sup> anion suggested that all dissolved W existed primarily in the form of the WO<sub>4</sub><sup>2-</sup> anion. Based on the percentage of W in the compound dissolved at 28 days and 1 mg/L loadings, the solubility rankings are: Na<sub>2</sub>WO<sub>4</sub>.2H<sub>2</sub>O ~ (NH<sub>4</sub>)<sub>10</sub>(H<sub>2</sub>W<sub>12</sub>O<sub>42</sub>).4H<sub>2</sub>O ~ (NH<sub>4</sub>)<sub>6</sub>(H<sub>2</sub>W<sub>12</sub>O<sub>42</sub>).4H<sub>2</sub>O > WO<sub>3</sub> > WO<sub>x</sub> > W metal > WC. For the tungsten carbides at pH 6, we were able to develop a regression correlation of seven-day WO<sub>4</sub><sup>2-</sup> concentrations to surface area loading: log (WO<sub>4</sub><sup>2-</sup>, micro g/L) = -2.2388 + log 1.0287 log (A, mm<sup>2</sup>/L) (n = 27; r<sup>2</sup> = 0.987) in which A is the measured surface area loading of tungsten carbide. We provide worked examples of how the T/D data could be used to derive hazard classification outcomes for these W compounds and metal. The T/D data enable producers to submit these classification outcomes to regulatory agencies to ensure compliance with environmental protection measures.

**234 Timing and Mechanism of Cell Death Induction and Alteration of Thymocyte Development upon Exposure to DES and Methoxychlor Metabolite, HPTE** C. Broussard, The Univ of La Verne, Univ of La Verne, Biology, Univ of La Verne, Professor of Biology; F. Mourad, The Univ of La Verne; P. Escalante, The Univ of La Verne, Biology; H. Johnson, D. Sortillon, C. Broussard, K. Pierce, The Univ of La Verne. Endocrine disrupting chemicals (EDCs) include a class of molecules that act as agonists or antagonists of the estrogen receptor or other hormone receptors. High production volume EDCs like pesticides and chemicals such as bisphenol A find their way back into the environment after their intended use. The prevalence of these chemicals poses a risk to aquatic and terrestrial organisms. Perhaps the most vulnerable populations are developing embryos, as events that occur during development can have long term consequences for the resulting adult organism. Studies in the last ten years have indicated that EDCs not only alter the reproductive system, the brain, and behavior, but may also impact immune responsiveness. We were interested in probing the mechanism of action of EDCs on the development of the immune system. Previously, we investigated the effects of diethylstilbestrol (DES) and hydroxyphenyl-trichloroethane (HPTE) on embryonic thymocyte development and found that both appear to induce cell death and alteration of differentiation. Here we report on a recent investigation of the timing and mechanism of DES- and HPTE-induced immune effects in an in vitro thymocyte differentiation assay. In the current study, thymocyte apoptosis was probed using Annexin V and PI staining, as well as a chromatin condensation assay, in cultures established with varied periods of exposure to EDCs. The signaling status of thymocytes was probed by CD5 surface staining. Results indicate a short exposure duration for induction of apoptotic effects and possible selective targeting of thymocyte populations for death. This research is supported by NIEHS ES017345-01. The opinions expressed in this work are solely the authors'.

**235 Associations of Perfluorochemicals (PFCs) with Immune, Biochemical and Hematological Parameters in Bottlenose Dolphins** P. Fair, NOAA, National Ocean Service; T. Romano, Mystic Aquarium; T. Hulsey, Medical Univ of South Carolina; J.S. Reif, Colorado State Univ, Dept of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences; G.D. Bossart, Georgia Aquarium; J. Adams, NOAA, National Marine Fisheries Service; M. Houde, Environment Canada; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, National Water Research Institute; C.D. Rice, Clemson Univ; A. Schaefer, Harbor Branch Oceanographic Institution at Florida Atlantic Univ; P.M. Margie, Univ of Las Vegas. Despite the widespread occurrence of perfluorochemicals (PFCs), very little is known regarding the impact of these contaminants on the health of wildlife populations. This study, therefore, investigated the relationship between PFCs and health/immune parameters in two populations of Atlantic bottlenose dolphins. A repeated cross-sectional comprehensive health assessment study was conducted during the summers of 2003-2005 near Charleston, SC (CHS; n=80) and the Indian River Lagoon, FL (IRL; n=82). Correlative data analysis stratified by site examined relationships between PFC levels in serum and immune, biochemical and hematology parameters. Significant relationships between immunological measures were observed with ΣPFC,

as well as individual PFCs including PFOS. B cell proliferation and numbers of CD2<sup>+</sup>, CD4<sup>+</sup>, CD19<sup>+</sup>, CD21 and MHCII<sup>+</sup> cells were positively correlated with PFC levels in CHS dolphins. Similar results were observed for IRL dolphins with two of these parameters: B cell proliferation and numbers of CD21<sup>+</sup> cells. PFC levels were also positively associated with lymphocyte numbers, CPK, ALP, cholesterol, TIBC, iron, and triglycerides and negatively with eosinophils in CHS dolphins. ALT, ALP, BUN, BUN/creatinine, Ca, CPK, and LDH were positively correlated with PFC levels in IRL dolphins. Serum proteins exhibited negative relationships with PFC concentrations. The number of significant associations with PFCs and the strength of these relationships were higher in CHS dolphins likely corresponding with their higher PFC concentrations. Bioaccumulation of PFC contaminants in dolphins may modulate immune responses and alter hematological and serum analytes and pose a threat to the health and viability of these populations.

**236 Immunotoxic Effects of Persistent Organic Pollutant Exposure on Juvenile Northern Leopard Frogs (*Lithobates pipiens*)** T.L. Cary, Univ of WI – Madison, Forest and Wildlife Ecology, Univ of WI-Madison, Zoology, Univ of WI-Madison, Forest and Wildlife Ecology; Y. Tsai, Univ of Wisconsin, Forest and Wildlife Ecology; W.H. Karasov, Univ of WI – Madison, Forest and Wildlife Ecology. Immunotoxicology is an emerging field and has the potential to increase knowledge regarding sublethal toxicity of contaminant exposure, including alterations in disease susceptibility. Worldwide presence of persistent organic pollutants such as polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB) have prompted increased interest in studying the potential immunotoxicity of these compounds. We conducted a dietary tadpole exposure study in order to assess how environmentally relevant levels of PBDEs impact the immune function of post-metamorphic frogs. Beginning at the free-swimming stage, *Lithobates (Rana) pipiens* tadpoles were exposed to DE-71, a pentabromodiphenyl ether mixture, at environmentally relevant levels (0, 1.0, 6.1, 71.4, 634 ng/g) through metamorphic climax. To assess how DE-71 exposure affects the adaptive immune response, we used an enzyme-linked immunosorbent assay (ELISA) to measure specific-IgY production in post-metamorphic frogs following immunization with keyhole limpet hemocyanin (KLH). Additionally, a similar ELISA method was used to determine total antibody production of both IgY and IgM isotypes. DE-71-exposed frogs had decreased secondary antibody response to KLH compared to unexposed frogs (p = 0.024), however, total IgY and IgM antibody production were not altered by PBDE exposure (p = 0.355 and 0.328, respectively). These findings lend evidence that larval exposure to PBDEs may influence specific antibody production in juvenile *L. pipiens*, but may not affect circulating levels of non-specific antibodies. To further analyze how persistent organic contaminants influence immune function in amphibians, we are currently conducting a dietary exposure to PCB-126, a known immune suppressor in other taxa. These results will be forthcoming, and discussed in comparison with the effects of DE-71 exposure.

**237 Epizootic Shell Disease in American Lobsters from Long Island Sound Associated with Reduced Immune Competency and not Contaminant Exposure** A. McElroy, Stony Brook Univ, School of Marine and Atmospheric Sciences; M. Homering, Skokomish Tribe; G. Taylor, B. Allam, Stony Brook Univ, School of Marine and Atmospheric Sciences. Epizootic Shell Disease (ESD) is known to affect American lobsters (*Homarus americanus*) along the northeast coast of the US forming erosional pits in the exoskeleton. ESD decreases the economic value of the lobster catch and in extreme cases can be lethal. First documented in 1997 in the waters off southern Rhode Island, prevalence and geographic distribution of disease incidence seems to be expanding. In New York waters, prevalence exceeds 20% of individuals in Eastern Long Island Sound (ELIS), but is barely detectable in the more contaminated waters of Western Long Island Sound (WLIS). As part of a larger consortium of researchers investigating the etiology of ESD in New England, we sought to assess immune capacity of lobsters with and without shell disease and the relative immune capacity of individuals from the ELIS and WLIS populations. Lobsters from Maine were also evaluated as a reference population. A large suite of defense factors were examined including: hemolymph antimicrobial activity and bacterial load, shell thickness, as well as a number of hemocyte parameters such as cell counts, phenoloxidase activity, reactive oxygen species production, and phagocytic activity. Despite high inter-individual variability in defense-related factors, multivariate analyses showed that lobsters from ELIS presented significantly reduced immune performance relative to lobsters from either WLIS



or Maine. Disease severity was negatively correlated with several immune parameters suggesting that the disproportionately high prevalence of ESD in ELIS may be related, at least in part, to reduced immunocompetency of ELIS lobsters. These data support the importance of evaluating multiple components of immune response when evaluating disease susceptibility, and suggest that despite significantly elevated exposure to contaminants, WLIS lobsters have developed resistance to ESD and possibly other infectious diseases. Funding was provided through RI Sea Grant and the Lobster Research Initiative.

**238 Immune Response of Rainbow Trout (*Oncorhynchus mykiss*) Coexposed to Benzo[a]pyrene and *Aeromonas salmonicida*** L.J. Phalen, N.S. Hogan, G.Z. MacDonald, K. Thorpe, M.R. Vandenheuvel, Canadian Rivers Institute, Univ of Prince Edward Island, Biology. Environmental toxicants such as polycyclic aromatic hydrocarbons (PAHs) are thought to be immunosuppressive. This study aimed to determine whether the use of four rainbow trout leukocyte-specific antibodies coupled with fluorescence assisted cell sorting analysis (FACS) could detect changes caused by the PAH benzo[a]pyrene (B[a]P) to the immune response elicited by rainbow trout in response to inactivated *Aeromonas salmonicida* (A.s.). Fish were exposed intraperitoneally to 2.5 or 25 mg B[a]P/kg BW simultaneously with formalin inactivated A.s. for 1, 3 or 7 days in two replicate trials. Absolute erythrocyte and leukocyte counts were measured in blood using a DiOC<sub>6</sub> staining technique coupled with FACS. Leukocytes were isolated from spleen, peritoneum, head kidney and blood, stained using fluorescently labelled antibodies to determine the proportions of B-cells, T-cells, thrombocytes and myeloid cells using FACS. Liver monooxygenase enzyme activity and fluorescent bile metabolites were used to evaluate exposure. Bile metabolites showed consistent exposure proportional to dose though there were differences in liver monooxygenase activity trends between trials. An approximate 25% decrease of the number of total leukocytes in whole blood was observed at high dose on day 7 only in Trial 1. A decrease in B-cell proportions was found at the high dose on day 3 and 7 in spleen (~10% each) and at low and high dose on day 7 in peritoneum (~16% and 21% respectively). A decrease in percentages of viable cells was found at high dose on days 3 and 7 in peritoneum (~13% and ~40% respectively) and head kidney (~4% and ~6% respectively). An approximate 26% increase in the number of myeloid cells was seen at high dose on day 7 in peritoneum. These results imply that leukocytes die at the site of injection in the peritoneum and that movement of B-cells from spleen populations could be resulting in splenic B-cell depletion.

**239 Titanium Dioxide Nanoparticles Modulate Innate Immune Response and Increase Mortality of Fish Exposed to Bacterial Pathogen** B. Jovanovic, Iowa State Univ, Biomedical Sciences; K. Kimura, Iowa State Univ; D. Palic, Iowa State Univ, Dept of Biomedical Sciences. Previously we have demonstrated that titanium dioxide nanoparticles (nano-TiO<sub>2</sub>) can change function of neutrophils in fish models both in vitro and in vivo. Neutrophils are primary sentries for bacterial infection in healthy individuals, and their role is to entrap and kill the invading pathogens. Here, we investigate how fish (*Pimephales promelas*), immunocompromised by nano-TiO<sub>2</sub>, respond to accompanying infection of *Aeromonas hydrophila* bacteria. Briefly, fish exposed to low environmentally relevant sublethal concentration of nano-TiO<sub>2</sub> coupled with *A. hydrophila* infection displayed 50-90% mortality of experimental population. On the contrary, control group exposed only to *A. hydrophila* have only 10% mortality, while nano-TiO<sub>2</sub> treated group and no treatment group do not show any sign of mortality or morbidity. The results are coupled with histopathology reports and ICP-MS analysis of nano-TiO<sub>2</sub> distribution in fish organism with particular outline on hematopoietic organs. In conclusion, environmentally relevant concentration of manufactured nano-TiO<sub>2</sub> has potential to obliterate fish populations by impairing their immune response toward bacteria during possible disease outbreaks in aquatic ecosystems. This is by our knowledge the first attempt in Science to understand the interplay between nanoparticles, immune system and bacterial infection in any animal model.

**240 Immunotoxicity of Atrazine and Nonylphenol in Rainbow Trout (*Oncorhynchus mykiss*): A Tale of Immune Function, Disease Challenge and Genomics** L.K. Shelley, Simon Fraser Univ, PHD student; P.S. Ross, Fisheries and Oceans Canada, Institute of Ocean Sciences; K.M. Miller, K.H. Kaukinen, Fisheries and Oceans Canada, Pacific Biological Station; C.J. Kennedy, Simon Fraser Univ, Dept of Biological Sciences. Microarray

technologies are increasingly being used to examine changes in gene expression in fish following exposure to either pathogens or chemicals individually, but have rarely been applied in immunotoxicological studies in which responses to both pathogen and chemical exposures are considered together. Atrazine and nonylphenol are commonly identified contaminants in aquatic habitats, however, relatively few studies have considered the potential impact of these suspected endocrine disruptors on immune function. Rainbow trout, *Oncorhynchus mykiss*, were exposed to either atrazine (555 µg/L) or 4-nonylphenol (18 µg/L) for four days, followed by a one hour immersion challenge with the bacterial pathogen *Listonella anguillarum*. Fish were then monitored for pathogen-related mortality for fourteen days. Blood and tissue samples taken before and after disease challenge revealed both contaminant and disease-related changes to plasma cortisol concentrations, hematocrit, leukocyte differential and spleen somatic index. In addition, 4-nonylphenol treatment increased mortality of fish three fold following the disease challenge. Liver samples from the control, atrazine and nonylphenol groups were used in 32K salmonid microarrays (cGRASP, 32K version 1) to assess genomic responses to both chemical exposure and chemical + disease challenge treatments. Results reveal significant changes in liver gene transcription following all treatments which may be related to the alterations in physiological indicators and the increase in disease susceptibility of treated fish. Overall, this study provides insight into the molecular mechanisms underlying the physiological effects of both atrazine and nonylphenol exposure in fish, with microarray technology providing considerable new insight into the immunotoxicity of contaminants in fish.

**241 Assessment of Immune Gene Expression and *Trypanosoma carassii* Infection in Goldfish Exposed to Naphthenic Acids and Oil Sands Process Water** M.O. Hagen, S. Mitchell, B.A. Katzenback, A. Oladiran, E. Garcia, M. Karpman, D. Beauparlant, Univ of Alberta, Biological Sciences; M. Gamal-El Din, D.W. Smith, Univ of Alberta, Civil and Environmental Engineering; J. Martin, Univ of Alberta, Laboratory Medicine and Pathology and Public Health Sciences; M. Belosevic, Univ of Alberta, Biological Sciences. The separation of bitumen from the oil sands generates large quantities of oil sands process water (OSPW), which cannot be released into the environment, until it is successfully remediated. In this study, we exposed goldfish for up to 12 weeks to either commercial NAs or aged OSPW using a real-time continuous flow exposure apparatus. We measured the gene expression of three pro-inflammatory cytokines (IFNγ, IL1-b1, and TNFα-2), anti-inflammatory cytokine IL-10, and pro-inflammatory cytokine receptors (IFNR1-1, IFNR1-2, TNFR1, TNFR2), in the gill, kidney, and spleen of the exposed fish using quantitative-PCR (qPCR). We observed a general up-regulation of IFNγ, IL1-b1, and TNFα-2 gene expression after acute (one week) exposure of fish to either commercial NAs or OSPW. Sub-chronic (12 weeks) exposure of fish to NAs caused a significant down-regulation in immune gene expression in fish exposed to higher doses (10 and 20 mg/L of NAs). In contrast, gene expressions in fish exposed to aged OSPW were similar to those of non-exposed controls. In general, there were no differences in gene expression of pro-inflammatory cytokine receptors between exposed and non-exposed fish except for the kidney, where the gene expressions of TNFR1 and TNFR2 were up-regulated and IFNG1-1 was down-regulated after sub-chronic exposure to OSPW. To determine whether goldfish host defense was affected by exposure to 5 mg/L and 20 mg/L of NAs, fish were infected with a blood parasite *Trypanosoma carassii* and their ability to control the infection and immune gene expression documented. After acute exposure to NAs (one week), fish exhibited significantly higher parasitemia compared to non-exposed controls. In contrast, after sub-chronic (60 days) exposure of fish to NAs, fish had higher parasitemia and greater mortality compared to non-exposed controls. Thus, the exposure of goldfish to NAs elicited a significant enhancement in pro-inflammatory gene expression and increased resistance to parasitic infection after acute exposure to NAs or OSPW, and down-regulation of immune gene expression and increased susceptibility to parasitic infection after sub-chronic exposure to NAs or OSPW.

**242 Fungicides: Shifts in Registration and Usage in the United States** G. Orrick, S. Hill, USEPA, Office of Pesticide Programs. Registration and usage of fungicides have shifted over the past decade. As a result, some new fungicides have become widely used, which may influence fungicide occurrence in water bodies. Fungicide usage patterns over the past ten years will be presented with a focus on the new fungicides introduced to the market during this time period. The environmental fate and toxicity properties

of historical and new fungicides will also be summarized, compared, and contrasted.

**243 Occurrence of Fungicides in Surface Waters Across the USA** K. Kuivila, K.L. Smalling, M. Hladik, J. Orlando, T. Reilly, US Geological Survey. Fungicides are pesticides designed to control fungal diseases, and tend to be understudied during routine monitoring of contaminants in aquatic environments. Used on a wide variety of crops, fungicides are typically applied repetitively throughout the growing season. Other uses include landscape maintenance, turf, and applications by homeowners. Chlorothalonil, a broad-spectrum fungicide, has been widely applied for over 50 years, but recently the registration and use of other new fungicides has been increasing. Most fungicides are moderately hydrophobic (log Kow of 2.5-4.5) and are persistent in water and sediments. To better understand the environmental occurrence of fungicides, methods were developed to analyze 34 current-use fungicides in water and sediments by using gas chromatography/mass spectrometry. Method detection limits for all compounds were in the low ng/L range for water and µg/kg range for sediments. In a series of studies from eight different states across the United States, surface-water samples (water n = 321 and sediment n = 89) were collected from a variety of agricultural-use settings (leafy greens, potatoes, rice, strawberries, and stonefruit orchards). Overall, twenty fungicides were detected in water and sediments. Two fungicides, azoxystrobin and boscalid, were detected primarily in water. Azoxystrobin was detected in 55% of water samples and at the highest concentration (128 µg/L), while boscalid was detected the most frequently (65% of samples) and at a maximum concentration of 3.60 µg/L. Three fungicides (chlorothalonil, myclobutanil, and pyraclostrobin) were found frequently in both water (detection frequency from 12 to 25%) and sediments (detection frequency from 15 to 27%). Tebuconazole was detected only in sediment (17% of samples) and at the highest concentration (1.38 mg/kg). Future studies, in collaboration with toxicologists, will include the analysis of fungicides in aquatic organisms to link measured tissue concentrations to potential effects.

**244 Fungicides and Current Use Herbicides in the Western Basin of Lake Ontario (Canada) Monitored Using POCIS Passive Samplers** C.D. Metcalfe, Trent Univ, Environmental & Resource Studies, Trent Univ, Dept of Environmental & Resource Studies; H. Li, Trent Univ, Water Quality Centre; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch. Fungicides are discharged into the aquatic environment with municipal wastewater and they are also transported into the aquatic environment in urban and agricultural runoff. In this study, we evaluated the spatial distribution of fungicides, as well as some current use herbicides in the near shore zone of the western basin of Lake Ontario and in urban creeks discharging into the basin by using passive sampling with the polar organic chemical integrative sampler (POCIS). Passive sampling provided estimates of time-weighted average concentrations of contaminants over a deployment period of 28 days. Sampling rates (Rs) of the target compounds into POCIS determined in static assays at 20°C ranged from  $0.293 \pm 0.032$  L/d for myclobutanil to  $0.648 \pm 0.042$  L/d for climbazole, except for Rs < 0.1 L/d for the herbicides, 2,4-D, dicamba and mecoprop and the fungicide, methyl-thiophanate. In POCIS samplers deployed at near shore stations and in urban creeks, the fungicides, azoxystrobin, myclobutanil and chloroneb, and the herbicide, dicamba were not detected at any of the monitoring sites. Carbendazim was detected in POCIS deployed at all stations; often accumulating to µg amounts over the deployment period. Several azole fungicides (fluconazole, propiconazole, tebuconazole, climbazole), as well as methyl-thiophanate and iprodione were also detected in POCIS. For all fungicides but methyl-thiophanate and carbendazim, the target compounds co-occurred with meprobamate, a persistent prescription pharmaceutical that was monitored in POCIS as an indicator of contamination by municipal wastewater. Note that clotrimazole, a widely used anti-fungal agent applied to the skin was only detected at one location, but this compound has been shown to be effectively removed by wastewater treatment. The presence of methyl-thiophanate, iprodione and carbendazim can probably be attributed to their use as anti-fungal biocides in agriculture and for the treatment of lawns and turf. The presence of several herbicides accumulated in POCIS in large amounts (i.e., atrazine, diuron, mecoprop) indicates that urban and/or agricultural runoff is the source of these contaminants. Time-weighted average concentrations of the fungicides and herbicides in water will be calculated using the Rs values determined in the laboratory.

**245 Fungicide Use in US Crop Production** M. Leggett, CropLife America; L. Gianessi, CropLife Foundation; W. Jones, CropLife America. A large majority of fruit and vegetable production in the US is reliant on the use of fungicides to protect them from destruction by plant pathogenic fungi. Fungicide applications, largely copper and sulfur have been a necessary part of agricultural production since the early 1900s. Synthetic fungicides have been applied to US crops since the early 1950's. Approximately 100 million pounds of fungicide are applied to US Crops annually in an ongoing struggle to stem the onset of plant disease. There are approximately 600 different compounds known as fungicides that can be grouped into 13 or more distinct classes with respect to mode of action. Of these, there are currently about 150 that are formulated and sold in commercial products globally. Application of compatible mixtures of fungicides is a common and recommended practice which is critical for effective disease control and to the maintenance of effectiveness of newer systemic and more specific fungicides since fungicide resistance can develop quickly if not well managed. The availability of varied disease control options facilitates effective management. Often non-chemical options for disease control are weak or non-existent, applications to some crops are often made repeatedly throughout the growing season. As with other pesticide compounds, the development process for fungicides includes extensive toxicological, fate and ecotoxicological testing prior to registration. In addition to current and changing patterns of use there are considerations in risk assessment that are somewhat unique to fungicides. New disease pressures and new patterns of use of fungicides are emerging that should be considered in the management of fungicides.

**246 Toxicity, Sub-lethal Effects and Potential Modes of Action of Select Fungicides for Freshwater Fish and Invertebrates** A.A. Elskus, US Geological Survey, S.O. Conte Anadromous Fish Research Laboratory; K. Kuivila, K.L. Smalling, US Geological Survey. Relatively few data are available on the effects of fungicides on non-target organisms in the aquatic environment, despite decades of agricultural and urban use. Given the basic modes of action through which fungicides exert toxic effects (mitosis and cell division, nucleic acids synthesis, respiration, amino acids and protein synthesis, signal transduction, lipids and membrane synthesis, sterol biosynthesis in membranes, glucan synthesis, melanin synthesis in cell wall, host plant defense induction, multi-site contact activity, with some whose mode of action is still unknown), one might expect these chemicals to be potent toxicants for all biological life. Indeed, an increasing number of studies report dramatic, and in some cases severe, effects of fungicides on fish and invertebrates at environmentally relevant concentrations. A series of complimentary studies are measuring the occurrence of fungicides in various use-settings across the USA. The fungicides selected for review are those detected most frequently and at the highest concentrations: azoxystrobin, boscalid, chlorothalonil, cyprodinil, fenarimol, fenbuconazole, fludioxinil, iprodione, myclobutanil, propiconazole, pyraclostrobin, pyrimethanil, tebuconazole, vinclozolin, and zoxamide. The presentation will review published literature, including striking fungicide effects on non-target organisms (fish and aquatic invertebrates), such as azoxystrobin effects on invertebrate community structure, fish respiration and reproduction, chlorothalonil alteration of fish immune function, the endocrine-disrupting activities of the P450-demethylase-inhibiting fungicides, and the presence and toxicity of boscalid, a recently introduced fungicide increasingly detected in waterways bordering agricultural settings. We also will discuss potential modes of action, sensitive organisms and life-stages, and data gaps.

**247 Fungicide-induced Declines of Freshwater Biodiversity Modify Ecosystem Functions and Services** J. Rohr, Univ of South Florida, Dept of Integrative Biology; T. McMahon, N. Halstead, L. Martin, T. Raffel, J. Romansic, Univ of South Florida; R. Boughton, Archbold Biological Station; P. Crumrine, Rowan Univ; S. Johnson, Univ of Florida. Despite the widespread use of fungicides, we know little about their impacts on freshwater ecosystems. Fungicides can be toxic to a variety of freshwater organisms, leading to possible negative effects on biodiversity and potential changes in ecosystem properties. However, research on indirect effects of contaminants on ecosystem properties is virtually nonexistent. We treated freshwater mesocosms with a solvent control or 1x or 2x the expected environmental concentration (EEC, ~164 µg/L) of chlorothalonil, the most commonly-used fungicide in the US, and quantified 34 community-level and 11 ecosystem-level responses to test for indirect effects of this chemical on ecosystem functions mediated by changes in biodiversity. We also conducted laboratory dose-response studies to examine how chlorothalonil influenced

survival, immunity, and corticosterone levels of amphibians, a highly imperiled freshwater group. In the mesocosms experiment, chlorothalonil did not significantly affect the survival of the macroarthropod community but the EEC increased the mortality of amphibians (nearly 100% mortality), gastropods, zooplankton, algae, and a macrophyte, resulting in a significant reduction in taxonomic richness. Chlorothalonil also significantly affected ecosystem functions and services, reducing decomposition rates, decreasing water clarity, elevating dissolved oxygen, and increasing net primary productivity as a result of algal blooms. A path analysis supported a model whereby both top-down (reduction in algal herbivores) and bottom-up (reduction in macrophytes) effects of chlorothalonil facilitated the algal bloom late in the experiment, which drove the shift in ecosystem functions. In the laboratory experiments, the -EEC caused 100% mortality of all four amphibian species tested within 24h, and 1/10000th of the EEC caused significant mortality of two species. Three species showed a non-monotonic dose-response, with low and high concentrations causing significantly greater mortality than intermediate concentrations and controls. For Cuban treefrogs, corticosterone exhibited a similar non-monotonic dose response and chlorothalonil concentration was negatively associated with liver tissue and immune cell densities ( $< 17.6\mu\text{g/L}$ ). This work emphasizes the need to further link contaminant-induced changes in biodiversity to altered ecosystem functions and to re-evaluate the safety of chlorothalonil to biodiversity.

**248 Effects of Fungicide Formulations on Ecosystem Function in Playas and Adjacent Native Grasslands and Croplands** S. Swain, Oklahoma State Univ, Zoology; L.M. Smith, Oklahoma State Univ; J.B. Belden, S.T. McMurry, Oklahoma State Univ, Dept of Zoology. Fungicides are used throughout the world to combat pathogenic fungi. They are commonly used on a large variety of crops and although they are meant to be used to fight fungal infections only when necessary, they are often applied throughout the growing season. The use of fungicides has dramatically increased in the past decade, particularly in the highly cultivated Great Plains of the USA. Fungicides, such as Quilt and Headline, which are relatively new classes of fungicides, are also EPA labeled to promote plant yield and health, leading to excess spraying of fungicides. In addition, runoff and drift during aerial application could contaminate surrounding landscapes, notably playas in the Plains area. The effects of Quilt and Headline on non-target fungal species such as beneficial decomposers have yet to be studied, specifically in playas of the High Plains (HP). Therefore, soil from playas and their adjacent uplands throughout the HP in native grassland and cropland will be collected and used to monitor effects on non-target fungal species by measuring ergosterol 7 and 14 days post fungicide application (at levels of 0, .1x, 1x and 10x the label rate). Ergosterol is a sterol only found in fungi and is a useful tool to measure fungal biomass and is targeted by propiconazole, an active ingredient in Quilt. In order to monitor mitochondrial processes, respiration will be measured using an alkaline trap method with an airtight container and NaOH capturing carbon dioxide evolved by the microorganisms. Similar to ergosterol measurement, respiration will be sampled with the same levels of fungicides. Both fungicides contain strobilurins whose mode of action targets fungal metabolism. Measuring respiration and ergosterol will monitor the effect of fungicides on the carbon cycling processes in HP playas, with emphasis on the difference between native grassland and cropland playa responses. Results will be presented on fungal biomass and respiration as these are key ecosystem functions affecting most ecosystem services.

**249 Sublethal Effects of Strobilurin Fungicides on Development and Growth of Larval Great Plains Toads (*Bufo cognatus*)** E.A. Hooser, S.T. McMurry, J.B. Belden, Oklahoma State Univ, Dept of Zoology; L.M. Smith, Oklahoma State Univ. Use of strobilurin fungicides has increased in the past few years to control disease outbreaks and in part, because of proclaimed plant yield benefits. As with most pesticides, drift and overspray during aerial applications, and runoff following storm events, can lead to contamination of non-crop areas, including wetlands embedded in agricultural fields. Pesticide concentrations at these off-site locations would be expected to be lower than concentrations intended for crop applications, and unlikely to cause mortality in non-target organisms. In this study we examined the sublethal effects of three strobilurin fungicides, Headline®, Stratego®, and Quilt®, on developmental rate and body size at metamorphosis of *Bufo cognatus* tadpoles. These metrics are good indicators of individual fitness and have implications for population viability. These endpoints are also relevant as the mode of action for the active ingredients is inhibition of cellular respiration and ATP acquisition. Thus, time to metamorphosis

and body size (growth) were chosen as they represent processes hindered by insufficient energy reserves. Tadpoles were exposed to sublethal concentrations of each fungicide continuously throughout the larval period, starting at Gosner stage 25 and terminating at stage 46. A replicated (n=6) static renewal system was used throughout the exposure period. Results from this experiment extend previous acute toxicity studies and provide the only known data on sublethal effects in an amphibian model.

**250 Biotransformation of Polyfluorinated Chemicals in the Environment – Thinking Beyond the Conventional Wisdom of Hydrocarbon Biochemistry** N. Wang, E.I. du Pont de Nemours & Co., Inc., E.I. du Pont de Nemours & Company; R.C. Buck, B. Szostek, P.W. Folsom, L.M. Sulecki, P.K. Cooper, J. Gannon, E.I. du Pont de Nemours & Company. Perfluorinated alkyl carboxylates (PFCAs) and sulfonates (PFSAs) are widely detected in the environment due to their broad and historical applications. The sources of PFCAs and PFSAs found in the environment include direct emissions and indirect precursor degradation. The 8:2 fluorotelomer alcohol (8:2 FTOH,  $\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$ ) is a key raw material used in the manufacture of FTOH-based products and its biotransformation leads to PFCAs and polyfluorinated carboxylic acids. Recently, 8:2 FTOH-based products and longer-chain analogs are being replaced with shorter chain length products based on 6:2 FTOH ( $\text{C}_6\text{F}_{13}\text{CH}_2\text{CH}_2\text{OH}$ ). Our work indicates that biotransformation of 6:2 FTOH and 8:2 FTOH in various environmental matrices (e.g., soil and activated sludge) leads to two major classes of transformation products: perfluorinated carboxylic acids and 4:3, 5:3 and 7:3 polyfluorinated acids [x:3 acids,  $\text{F}(\text{CF}_2)_n\text{CH}_2\text{CH}_2\text{COOH}$ ,  $n = 4, 5$  or  $7$ ]. The pathways leading to PFCA formation and x:3 acid further degradation by microbes in the environment are markedly different and much more complex than the ones predicted based on the molecular structures of 6:2 FTOH, 8:2 FTOH, and x:3 acids as well as metabolic pathways of hydrocarbon analogs. The PFCA formation and x:3 acid further degradation are via novel “one-carbon removal mechanisms” instead of microbial  $\alpha$ - and  $\beta$ -oxidation. The first key step for  $\alpha$ - and  $\beta$ -oxidation is to activate a polyfluorinated acid by acyl-CoA synthetase to form a thioester such as 5:3 acid-CoA ester, followed by four enzymatic steps to form corresponding one- or two-carbon shorter fluorinated acids. However, our experimental evidences with high-resolution mass spectrometry analysis show that polyfluorinated acids such as 5:3 acid cannot be activated by acyl-CoA synthetase for subsequent  $\alpha$ - and  $\beta$ -oxidation. Instead, 5:3 acid can be directly decarboxylated to form one-carbon shorter 4:3 acid via “one-carbon removal mechanisms”. This is the first report that carboxylic acids can be decarboxylated without the need to be activated by acyl-CoA synthetase. The multiple carbon-fluorine bonds of 5:3 acid prevent it to be activated but perhaps also provide intrinsic free energy for the decarboxylation without activation leading to 5:3 acid further degradation. The details of the “one-carbon removal mechanisms” and bioenergetics of 5:3 acid biotransformation will be discussed.

**251 Concentrations and Biomagnification of Perfluorinated Compounds in Lake Food Webs in the Canadian High Arctic** G. Lescord, Canadian Rivers Institute, Univ of New Brunswick, Biology Dept; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; D. Muir, A. De Silva, M. Williamson, C. Spencer, Environment Canada. Perfluorinated compounds (PFCs) can be found in air, sediment, seawater and biota across the Canadian High Arctic, however previous studies on PFC biomagnification have focused on the marine systems. Due to the remote location of Arctic systems and the relative stability of PFCs, it is hypothesized that Arctic lakes receive most of their PFCs from long range transport and atmospheric deposition. Once PFCs are deposited onto these lakes, they are bioaccumulated by lower trophic level organisms and biomagnify up the food webs to Arctic char. The main goal of this study is to determine the concentrations and biomagnification of various PFCs in the food webs of six lakes on Cornwallis Island, Nunavut. Two of these lakes (Meretta and Resolute) are located next to an airport, a local point source of contamination due to the past use of PFOS compounds for fire fighting. The remaining four lakes receive all PFC inputs atmospherically, offering a unique opportunity to compare the two contaminant sources within the same region. The six lakes were sampled repeatedly for sediments, invertebrates, and fish during and after the annual snow melt in July 2010 and 2011. Water samples were collected periodically to assess temporal changes in PFC concentrations. All samples were analyzed for a range of PFCs and the food web was characterized using stable isotopes; nitrogen isotopes were



used to assess trophic level and carbon and sulfur isotopes were used to assess energy source. Rates of PFC biomagnification through the food web will be quantified and compared among lakes through regression analysis. PFOS was the dominant PFC in adult char from all lakes. The mean PFOS detected in Meretta and Resolute Lake char muscle was 30 fold higher than in the four atmospherically supplied lakes (32.42 vs. 0.79 ng/g wet weight). Other notable PFCs in the char were PFNA and PFOSA (means of 0.72 +/- 0.21, 0.79 +/- 0.27 ng/g, respectively) in Meretta Lake and PFPeA and PFNA (means of 0.21 +/- 0.08, 0.16 +/- 0.07 ng/g, respectively) in fish from the four remote lakes. In water, mean PFOS concentrations were 300 times greater in Meretta and Resolute Lakes than in the other four lakes (25,021 ng/L vs. 77 ng/L, respectively). In the atmospherically contaminated lakes, the dominant PFC was PFOA (mean of 265 +/- 39 ng/L). All other PFC analyses sediments and biota are ongoing. Results from this study will improve our understanding of the concentrations of PFCs in Arctic lakes and offer critical baseline information for future studies.

**252 Dietary Bioaccumulation of Perfluorinated Phosphonates (PF-PAs) and Perfluorinated Phosphinates (PFPIAs) in Juvenile Rainbow Trout, *Oncorhynchus mykiss*** H. Lee, Univ of Toronto, Chemistry, Univ of Toronto, Dept of Chemistry, Univ of Toronto; S.A. Mabury, Univ of Toronto, Dept of Chemistry; A.O. De Silva, Environment Canada, Water Science & Technology Directorate, Environment Canada, Water Science and Technology. Perfluorinated phosphonates (PF-PAs) and perfluorinated phosphinates (PFPIAs) are high-production volume fluorinated surfactants used as leveling and wetting agents in waxes, coatings, and cleaning fluids. Recent observations of the PFPIAs at sub µg/L concentrations in human sera confirm human exposure to commercial products containing these fluorinated surfactants. Waste release of such products from domestic, commercial, and industrial sources may contribute to the load of these chemicals in aquatic systems. In fact, the PF-PAs were observed in Canadian surface waters and wastewater treatment plant (WWTP) effluents, while the PFPIAs were observed in WWTP sludge. The present study examines the bioaccumulation potential of PF-PAs and PFPIAs in juvenile rainbow trout (*Oncorhynchus mykiss*) through dietary exposure under flow-through conditions. Fish were administered a daily dose (1.5% body weight) of commercial fish feed containing a mixture of C6, C8, and C10 PF-PAs or a mixture of C6/C6, C6/C8, and C8/C8 PFPIAs, all prepared with authentic standards provided by Wellington Laboratories (Guelph, ON). Control fish were given non-dosed feed. Whole body concentrations were determined by extracting and analyzing whole fish in triplicate at timepoints during the 25-day exposure and 30-day depuration phases to calculate analyte-specific bioaccumulation factors, elimination rate constants and half-lives. The relationship between substitution at the phosphonate and/or phosphinate headgroup and extent of bioaccumulation will be explored through comparison of bioaccumulation factors calculated for each PFPA and PFPIA congener. The important role of the acid headgroup in driving the differential accumulation of perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSA) in biological tissues has been previously reported. PFSA have been demonstrated to bioaccumulate to a greater extent than their PFCA analogs of equal perfluorinated chain length in rainbow trout. The bioaccumulation potential of PF-PAs and PFPIAs, also perfluorinated acids, is predicted to vary with headgroup and chain length.

**253 Investigating the Biological Fate of Fluorotelomer Unsaturated Acids and Aldehydes** A. Rand, Univ of Toronto, graduate student, Univ of Toronto, Chemistry; S. Mabury, Univ of Toronto. Fluorotelomer unsaturated acids and aldehydes (FTUCAs and FTUALs) are intermediate metabolites that form from the biotransformation of fluorotelomer alcohols (FTOHs), found as impurities in commercial fluorinated surfactants and polymers. Studies have shown that these commercial products can also biologically transform to yield FTOHs, which further metabolize to ultimately produce PFCAs. PFCAs are persistent and potentially toxic, however, previous work suggests that the PFCA precursors may actually be more toxic than PFCAs themselves. The purpose of this current study was to assess the reactivity of FTUCAs and FTUALs with model proteins. In vitro experiments were carried out in an aqueous buffer system (pH 7.4), where FTUCAs and FTUALs of varying fluorinated chain lengths were reacted with apomyoglobin and human serum albumin to determine 1) whether these metabolites react with nucleophilic residues within proteins and 2) their covalent binding stoichiometry, using direct injection time-of-flight mass spectrometry (TOF-MS). The FTUALs formed one adduct with apomyoglobin while in

comparison, the FTUCAs were not reactive. This finding demonstrated the selectivity of FTUCAs towards biological nucleophiles containing cysteine. Previous work determining the reactivity of the unsaturated intermediates with amino acids also showed that reaction rates depended upon the amino acid: cysteine >> histidine > lysine >> arginine. FTUCAs and FTUALs were also spiked into in vitro cellular systems to determine the extent of protein binding in the presence of these reactive intermediates. This is the first study which probes the reactivity of FTUCAs and FTUALs with nucleophiles other than thiol groups, further elucidating their fate within biological systems.

**254 Perfluorinated Compounds in a US National Effluent Sampling** S.F. Nakayama, National Institute for Environmental Studies, Center for Environmental Health Sciences; M.A. Mills, USEPA, National Risk Management Research Laboratory; R. Marfil-Vega, USEPA, ORISE Research Participant to the National Risk Management, Univ of Cincinnati; K. Tadele, US Environmental Protection Agency, National Risk Management Research Laboratory; A.L. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; M. Kostich, USEPA Office of Research and Development, EERD; J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch. Mounting evidence suggests that municipal wastewater is one of the primary sources of perfluorinated compounds (PFCs) to the aquatic environment. As there is no treatment technology to date proven to efficiently remove the PFCs during drinking water treatment, it is extremely important to characterize nationally representative wastewater effluents in order to evaluate the extent of PFC occurrence and assess their impact on ecosystem and humans. Fifty large municipal wastewater treatment plants in the United States were identified from the 2004 Clean Watershed Needs Survey. These plants produce about 16% of all the municipal wastewater in the US. A one-time grab or 24-h composite sample (total of 9 litres) of finished effluent from each plant were collected by EPA Regional or Plant personnel, then shipped to EPA – Cincinnati laboratory. For each effluent sample, the concentration of perfluorinated compounds were quantified along with selected pharmaceuticals, steroid hormones, alkylphenolic compounds, Bisphenol A, and the estrogenic potential in a fish assay. For PFCs analysis, solid phase extraction method was employed coupled with liquid chromatography tandem mass spectrometry. Perfluorinated compounds were found in most of the effluents in a wide range of concentrations. The result from the national effluent study will be discussed in the presentation.

**255 Sources of Perfluorooctanoate in Remote Locations** J.P. Benskin, AXYS Analytical Services Ltd; L. Ahrens, Environment Canada, Science And Technology Branch; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute; C. Spencer, Environment Canada, Aquatic Ecosystem Protection Research Division; B. Rosenburg, Fisheries and Oceans Canada, Arctic Aquatic Research Division; G. Tomy, Dept of Fisheries & Oceans Canada, Dept of Fisheries & Oceans; H. Kylin, Swedish Univ of Agricultural Sciences, Aquatic Sciences and Assessment; R. Lohmann, Univ of Rhode Island, Graduate School of Oceanography, Univ of Rhode Island; V. Phillips, Univ of Regina, Dept of Biology; V. StLouis, Univ of Alberta, Biological Sciences; A.O. De Silva, Environment Canada, Water Science & Technology Directorate, Environment Canada, Water Science and Technology; J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine and Pathology. The extent to which historical (i.e., electrochemically fluorinated) and contemporary (i.e., telomer) manufactured perfluorooctanoate (PFOA) contribute to concentrations in remote environments is widely debated. So too is the dominant mechanism of long-range transport. Here, relative contributions of historic and contemporary-manufactured PFOA were assessed in seawater samples from the Arctic, Atlantic, and North and Norwegian Seas, as well as in dated alpine sediment cores from the Canadian Rockies, using an established isomer profiling technique. Eastern Atlantic PFOA was largely (83 – 98%) historic; but this decreased to as little as 33% close to the Eastern US seaboard. PFOA in the Norwegian Sea was also near exclusively historic, but the relative contribution decreased to ~50% near the Baltic Sea. In the Arctic, a spatial trend was observed whereby PFOA in seawater originating from the Atlantic was predominantly of historic origin (up to 99%), whereas water in the Archipelago (i.e., from the Pacific) was predominantly of contemporary origin (as little as 17%

historic). Perfluorinated carboxylate isomer profiles (C7-C13 perfluoroalkyl chain lengths) in dated alpine sediment cores were exclusively linear and temporal trends from the 1950s-2008 were consistent with fluorotelomer emissions estimates over this time. Taken together, these results suggest that atmospheric deposition of contemporary-PFOA, or related precursors, is the dominant source of PFOA to the Canadian Arctic Archipelago (and western Arctic), while historically manufactured PFOA and direct oceanic transport is the dominant source of PFOA to the North Atlantic Ocean. Observations of contemporary PFOA in coastal regions, coinciding with elevated PFOA concentrations, suggests that continued production of PFOA is increasing the burden of this contaminant in the world's oceans.

**256 Spatial Distribution and Loadings of Particle Sorbed and Dissolved Perfluorinated Compounds in the Basin of Tokyo Bay** Y. Zushi, National Institute for Environmental Studies, Center for Environmental Measurement; F. Ye, Yokohama national Univ, Graduate School of Environment and Information Sciences; M. Motegi, K. Nojiri, S. Hosono, Center for Environmental Science in Saitama; T. Suzuki, Y. Kosugi, K. Yaguchi, Tokyo Metropolitan Institute of Public Health; S. Masunaga, Yokohama national Univ. A number of studies on perfluorinated compound (PFC) pollution in water environment have been reported. However, the information on many types of PFCs including their partitioning behavior in water environment is limited. We analyzed over 30 types of PFCs including precursors both in dissolved phase and particle solid phase in 50 samples of river water collected from entire part of Tokyo Bay basin. PFCs were detected from suspended solids (SS) with the range of < 0.003 – 4.4 ng/L (0.11 – 2470 ng/g-dry SS). The concentrations of PFCs in SS (ng/L) were one to two order(s) of magnitude lower than that of PFCs in dissolved phase. The predominant PFCs in SS were PFOS (median: 0.24 ng/L), PFNA (median: 0.17 ng/L) and PFUnDA (median: 0.12 ng/L). Longer chain PFCAs (C12 – C17) were frequently detected above LOQ from SS samples (56 – 80%) whereas those in dissolved phase were lower than LOQ (> 60% samples) in most samples. Relatively high levels of PFCs (total of 35 PFCs) in SS were observed in urbanized area, such as building and traffic area. The concentration of PFCAs including PFOA and PFNA were significantly correlated with the geographic index such as artificial area (R<sup>2</sup> of linear regression curve in double logarithmic plot: 0.09 – 0.55). On the other hand, PFOS and FOSA were significantly correlated with arterial traffic area (R<sup>2</sup> in double logarithmic plot: 0.29 – 0.55). Those spatial trends were similar with the case in dissolved PFCs, though R<sup>2</sup> in regression analysis were lower compared with the case in dissolved PFCs. The dissolved PFCs were more appropriate indicator to obtain the information on PFC sources. We have estimated the loadings of PFCs into Tokyo Bay from 6 main rivers and found that more than 90% of total PFCs flowed into Tokyo Bay as dissolved phase. However, 40.0 – 83.5 % of long chain PFCAs, such as PFDoDA, PFTTrDA, PFTeDA and PFPeDA were transported as particle phase. Rain runoff event might increase the loadings of PFCs in SS. We should pay more attention to particle phase PFCs, especially for longer chain PFCs.

**257 Temporal Changes Over Twenty Years of Perfluoroalkyl Sulfonates and Carboxylates in Herring Gull Eggs from Sites Across the Laurentian Great Lakes** W.A. Gebbink, Carleton Univ, Dept of Chemistry; R.L. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environment, Canadian Wildlife Service; C.E. Hebert, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division; C. Weseloh, Environment Canada, Environment Conservation Branch. We investigated the temporal trends of perfluorocarboxylic (PFCA) and sulfonic acids (PFSA) and selected precursors in herring gull eggs from seven colony sites across the Laurentian Great Lakes of North America. The herring gull eggs collected in 1990 and 1997 to 2010 (1 pool of 13 eggs per year per site) were screened for PFCAs (C6-C15), PFSA (C4, C6, C8, C10), fluorotelomer unsaturated acids (6:2, 8:2, 10:2 FTUCAs), fluorotelomer alcohols (6:2, 8:2, 10:2 FTOHs) and perfluorosulfonamides (PFOSA and NMeFOSA). Declines in  $\Sigma$ PFSA concentrations occurred in egg pools from all the colonies, which was significant for Agawa Rocks (Lake Superior) and Channel-Shelter Island (Lake Huron); however, concentrations increased significantly for Toronto Harbour (Lake Ontario). Perfluorooctane sulfonate (PFOS) dominated the PFSA pattern in the eggs at all colonies and all years, ranging from 79.9 to 99.4%. Declining concentrations of PFOS in the eggs from most colonies could be related to

the phase out of PFOS and its precursors in 2002 by the 3M Company; however, the increasing concentrations at Toronto Harbour might be a result from being close to highly urbanized area. PFOSA (PFOS precursor) was detected in the eggs between 1990 and 2006; post-2006 PFOSA was below detection limit at all the colonies. Declining concentrations were observed between 1990 and 2006 at all the colonies with the exception of Agawa Rocks and Channel-Shelter Island. The  $\Sigma$ PFCA concentrations increased significantly in the eggs from Agawa Rocks, Fighting Island (Detroit River), Niagara River, and Toronto Harbour. The eggs from Gull Island (Lake Michigan), Channel-Shelter Island and Chantry Island (Lake Huron) showed no significant changes during 1990 and 2010. The PFCA pattern was dominated by PFDA, PFUnA, PFDoA, and PFTTrA, and generally the patterns were consistent among years and colonies. PFCA precursors (FTOHs and FTUCAs) were below detection limit in the egg pools from all years and colonies. Environmental degradation of these precursors could explain the increasing trends of PFCAs as production volumes of these telomer based compounds are still increasing. Declining concentrations of the C8 PFOS, PFOA and PFOSA, might be a result of the 2002 phase out of the C8 chemistry by 3M in North America. However, increasing production volumes of fluorotelomer based compounds, and degradation of these compounds to PFCAs might explain increasing trends of PFCAs.

**258 Hydrocarbons and Legacy Organochlorine Pesticides and PCBs in Sediments from Terminos Lagoon, Mexico** B. Ramirez-Vargas, EPOMEX, Universidad Autonoma de Campeche; E. Cach-Perez, Campeche Autonomous Univ/EPOMEX Center; L. Alpuche Gual, Campeche Autonomous Univ/EPOMEX Center, Centro Epomex; G. Gold-Boucher, Cinvestav Unidad Merida, Marine Resources. As a part of a pilot project to enhance Mexico's capabilities for coastal monitoring a sampling was carried out in Terminos Lagoon, in the Southern Gulf of Mexico. This is Mexico's largest coastal lagoon, located at the estuary of the Grijalva-USumacinta river system that accounts for 35 % of total freshwater runoff in Mexico and is the second in the Gulf of Mexico. A total of 60 sampling stations were planned in a probabilistic design, stratifying in six subareas using the hydrological and sedimentological characteristics of the lagoon. Fish, water and sediments were collected for a wide variety of chemical and biological analyses, but here only the results for hydrocarbons and pesticides in sediments are presented. Results indicate that there are significant differences between the six areas, and that the number of sampling stations can be decreased. Results are discussed in terms of the fraction of stations exceeding the ERL and ERM guidance values. This is important for coastal management.

**259 Levels and Distribution of Polychlorinated Biphenyls In The Vicinity of Chinese Great Wall Station, Antarctica** Q. Zhang, Y. Li, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, State Key Laboratory of Environmental Chemistry and Ecotoxicology; P. Wang, D. Geng, G. Jiang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The Antarctica is considered to be a clean and pristine area due to less anthropogenic activities. However, more and more studies have reported the detection of various POPs in the Antarctic environment. In this study, various environmental samples including air (high-volume air samples and PUF-disk passive air samples), soil, sediment, lichen and moss were collected in the vicinity of Chinese Great Wall Station on the King George Island, Antarctica, in the austral summer between December 2009 and February 2010, to investigate the levels and distribution of polychlorinated biphenyls (PCBs) in this remote area. The analysis of PCBs was performed using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS) with an isotope-dilution method. The air concentrations of total PCBs ranged between 82-167 pg m<sup>-3</sup> from high volume air sampling, and were dominated by light homologues (Di to Tetra-CBs). There was a 1-3 fold deviation of PCB concentrations between the active and passive sampling data. Therefore, PUF-disk passive air sampling can be used to assess PCBs distributions in the Antarctica atmosphere, although the wind speed are always high during the sampling campaigns. The three sampling sites closing to several Antarctic Research Stations showed higher PCB concentrations than the other two sites, which may reflect potential influence from human activities. In the soil and sediment samples, PCB concentrations were in the range of 60.1-1436 pg g<sup>-1</sup> dry weight (dw). In the lichen and moss, they were between 404-745 pg g<sup>-1</sup>dw and 406-952 pg g<sup>-1</sup>dw, respectively, of which no significant difference was observed between the grass types. The most abundant isomers were the lower chlorinated CBs in all the samples except

for the dropping-amended soils from Ardley Island, where HxCBs dominated. Furthermore, the indicator CBs, CB-118, -138 and -153 were of high concentrations besides CB-28 in all the samples, which might indicate local source exist (e.g., biotic activities). Notably, CB-11 was significantly detected, the higher ratio of CB-11 in the natural soil than in the dropping-amended soil suggested different source from biotic activities. Therefore, the contamination profiles indicated long-range atmospheric transport could be the main source of PCBs in King George Island; however, it was also apparently influenced by local sources (e.g., research station, tourism and biotic activities).

**260 Polycyclic Aromatic Hydrocarbons in Continental Shelf Sediment of China: Implications for Anthropogenic Influences on Coastal Marine Environment** L. Liu, Guangzhou Institute of Geochemistry; J. Wang, Health Canada; Y. Guan, South China Normal Univ; E.Y. Zeng, Guangzhou Institute of Geochemistry, State Key Lab of Organic Geochem. Sediment samples collected from the continental shelf of China, embracing Yellow Sea, inner shelf of the East China Sea, and the South China Sea, were analyzed for polycyclic aromatic hydrocarbons (PAHs), from which the potential influences of terrestrial anthropogenic activities on the coastal marine environment were assessed. The concentrations of anthropogenic PAHs ( $\Sigma 19\text{PAH}$ , List S1 in the Supporting Information) were 27\_220 ng/g dry weight, with a mean of 90 ng/g. An alongshore decreasing trend of  $\Sigma 19\text{PAH}$  concentrations was observed in the inner shelf sediment of the East China Sea, suggesting fluvial input was the major source of PAHs in the region. On the other hand, sediment PAHs in Yellow Sea and the SCS were principally derived from atmospheric deposition. Compositional pattern analyses suggested that Yellow Sea sediments were dominated by high molecular weight (5-6-ring) PAHs, accounting for 50\_72% (average: 60%) of total PAHs, whereas low molecular weight PAHs contributed to 42\_96% (average: 66%) of total PAHs in the South China Sea sediment. This spatial variability of PAH compositions was ascribed to the regional climatic divergence and/or regional source profiles. The generally higher  $\Sigma 19\text{PAH}$  levels (27\_220 ng/g with a mean of 110 ng/g) in the Yellow Sea sediment than those in the SCS sediment (28\_210 ng/g with a mean of 75 ng/g) were probably resulted from larger emissions of PAHs from domestic coal combustion and coke industry in North China than in South China.

**261 Spatial Variability of Factors Influencing the Distribution of Triclosan in Sediments and Water of an Urbanized Estuarine Embayment** D.R. Katz, M. Cantwell, J. Sullivan, USEPA, Atlantic Ecology Division; M. Perron, Brown Univ, School of Engineering; M. Charpentier, Raytheon Company; R.M. Burgess, USEPA, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, USEPA. Triclosan (TCS) is a broad spectrum anti-microbial compound added to many consumer and personal care products. TCS enters water bodies primarily through wastewater treatment plant (WWTP) effluent and may be introduced by combined sewer overflows or surface water runoff. In estuarine waters, TCS adsorbs onto particles and may be preserved for long periods of time after deposition to sediments. The spatial variability and factors influencing deposition of TCS in marine ecosystems are the subjects of this research with a focus on Greenwich Bay, a sub-estuary of Narragansett Bay, Rhode Island. Sample locations were chosen using a statistically randomized, tessellated hexagonal grid design. After extraction and cleanup, sediment and water samples were derivatized and analyzed by GC/MS-EI. Overall, results show a high correlation to the sediment organic carbon content ( $r^2 = 0.79$ ). Locally elevated sediment concentrations of TCS in Greenwich Cove, which directly receives effluent from a local WWTP, provide evidence that it is a major source of TCS to the area. Dissolved water concentrations were spatially variable, suggesting there may be other discrete sources of TCS to Greenwich Bay, or that additional factors outside the sub-embayment may have a role in TCS flux to this area. A sediment core collected from the center of the bay shows a sustained decline in TCS concentration from approximately 1990 to the present. This corresponds with increases in sewer connections and improvements in WWTP technologies employed in the watershed.

**262 Quondam Times Orchardring Creates Modern DDT-Problems** A. Ruus, Norwegian Institute for Water Research (NIVA), Norwegian Inst. for Water Research, N.I.V.A., Norwegian Inst. for Water Research; N. Green, NIVA; A. Maage, NIFES; C.E. Amundsen, Bioforsk; M. Schøyen, J. Skei, NIVA. The Sørkjørd (Western Norway) has a long history of agriculture

(since the 13th century), as well as industry (since the early 20th century). DDT was used as an insecticide in the orchards along the fjord shortly after World War II and until 1970, when it was banned as a general pesticide in Norway. Environmental concerns followed later and The Norwegian State Pollution Monitoring Program has been continuous in the fjord since 1979. It has inter alia comprised analyses of different contaminants in mussels, fish and sediments. Since the early 1990s, elevated concentrations of DDT were found in mussels and fish. Furthermore, on some locations DDT-concentrations in mussels increased towards present day, despite the discontinuation of use. In 2006, the highest concentrations were encountered so far, corresponding to approximately two orders of magnitude higher concentrations than what is regarded as a Norwegian background. Analyses of sediment core sections also showed increased input of DDT to the fjord towards present day. Shifts in climatic parameters (e.g., precipitation), as well as a decline in atmospheric sulphate deposition, followed by increased amounts of soil dissolved organic carbon, may have contributed to these observations. These findings show that although agreements and regulation of hazardous substances decrease or terminate their release to the environment, other factors may come into play that counter the effect of lowered concentrations in environmental samples.

**263 The Fate of Organochlorine Contaminants in Seabirds from an Arctic Colony: Bioaccumulation, and Transfer to Coastal Nesting Sites and Predators** K.L. Foster, Trent Univ, Dept of Chemistry; M.L. Mallory, Environment Canada, Canadian Wildlife Service; L. Hill, J.M. Blais, Univ of Ottawa, Program for Chemical and Environmental Toxicology. Seabirds accumulate contaminants from the prey that they consume, and thus are frequently used as biomonitors of marine contaminants. Seabirds also serve as a contaminant link or vector of marine-derived contaminants to coastal, terrestrial regions near their nesting sites, where they deposit these contaminants in their carcasses, partially digested food and excreta. In the present study, the bioaccumulation of organochlorine contaminants by northern fulmars (*Fulmarus glacialis*) from a colony at Cape Vera, Devon Island in the Canadian High Arctic (76°15'N, 89°14'W), and the subsequent transfer of these accumulated contaminants to coastal environments and terrestrial predators of the birds, are examined. Measured organochlorine concentrations and associated fugacities are presented for the liver, carcasses, and digesta collected along sections of the digestive tract including prey. Segmenting the digestive tract enabled a snap shot of contaminant fate within the birds, including contaminant uptake from prey items into the bird, and contaminant metabolism, through stages of digestion. Carcass contaminant concentrations are seldom monitored for seabirds, yet we have demonstrated their utility in assessing the transfer of marine contaminants to terrestrial predators such as the Arctic fox (*Alopex lagopus*). Elevated concentrations of contaminants in avian stomach oils, which are used to feed chicks and for defensive purposes, increase chick exposure and are relevant to bioaccumulation in young seabirds. The benefits of considering both concentrations and fugacities are demonstrated and provide information on the absorption and distribution of chemicals within the fulmars and contaminant transfer to offspring and predators.

**264 Quantitative Determination of Pharmaceutically Active Compounds in Singapore's Marine Environment** S. Bayen, National Univ of Singapore, Dept of Civil and Environmental Engineering; H. Zhang, National Univ of Singapore, Dept of Civil and Environmental Engineering, National Univ of Singapore; M. Desai, National Univ of Singapore, Tropical Marine Science Institute; B.C. Kelly, National Univ of Singapore, Dept of Civil and Environmental Engineering. The discharge of pharmaceutically active compounds (PhAC's) into the environment via household, municipal and industrial wastes is a pressing public health issue. Some of these chemicals have been reported as toxic to aquatic organisms in the literature and high environmental concentrations may cause toxic effects. Here, we present the development of analytical methods for the quantitative determination of several PhAC's in surface waters, sediments and biota from Singapore's marine environment. Target analytes include common human pharmaceuticals such as Atorvastatin, Carbamazepine, Citalopram, Diltiazem, Diphenhydramine, Fluoxetine, Norfluoxetine, Propanolol, Sertraline, Thiabendazole, Trimethoprim, Venlafaxine, Warfarin and Gemfibrozil. We conducted a comprehensive field survey of surface waters, sediments and organisms at various locations from Singapore's coastal marine environment. Seawater samples were filtered/extracted using a filtration apparatus containing glass fibre filters and solid-phase extraction (SPE) disks. Both acidic (pH=2) and



neutral (pH=7) extractions were tested and evaluated. We report distinct performances of each extraction protocols for the various analytes. Marine sediments and tissue samples were extracted using solvent extraction followed by SPE. Identification and quantification of target compounds was performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).  $^{13}\text{C}$  or deuterated mass-labeled standards were used for quantification (isotope dilution). Results of analyte recovery tests, procedural blanks and replicate sample analyses demonstrate generally good performance of the methods for the majority of target analytes. We encountered significant matrix effects (ionization suppression and enhancement), highlighting the need for careful quality control/assurance and utilization of mass-labelled surrogate standards. The significance of the occurrence, levels and patterns of various PhACs in Singapore's marine environment are discussed.

**265 Development and Evaluation of a Multimedia Environmental Fate Model for Phthalate Esters in the False Creek, British Columbia** G. Zandpoor, Simon Fraser Univ, Dept of Biological Science, Simon Fraser Univ; H. Lai, Simon Fraser Univ; M.G. Ikononou, Dept of Fisheries and Oceans, Institute of Ocean Sciences; F.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment), Simon Fraser Univ. Di-phthalate esters (DPEs) are widely used commercial chemicals. Their main application is as plasticizers. DPEs are high volume production substances that have been observed to be present in environmental media from many locations around the world. There is concern over the potential impacts of di-phthalate esters and their main metabolites, the mono-alkyl phthalate esters (MPEs), in wildlife and humans. This concern is partly caused by the lack of a quantitative understanding of the environmental fate of these chemicals in the environment. To improve the understanding of the relationship between the release of DPEs into environment and resulting concentrations of DPEs and MPEs in abiotic and biotic media, we developed and tested a multimedia environmental fate and food web model to describe the distribution and transformation of DPEs and MPEs in an aquatic ecosystem. To test the model, we conducted a field study of the distribution of DPEs and MPEs in a marine Inlet in Vancouver, Canada. In this presentation, we present the model, describe the field study and discuss the performance of the model. We demonstrate that the calculated sediment water partition coefficients (organic carbon normalized) are in good agreement with the corresponding independently measured values for both DPEs and MPEs. The only exception was dimethyl phthalate (DMP). Both model calculated and observed bioaccumulation factors of DPEs and MPE in fish and benthic invertebrate species are also presented. The model was applied to assess the combined loadings of DPEs into the Inlet and to assess the real-world half-life time of DPEs and MPEs in an aquatic environment. The multimedia mass balance environmental fate model in conjunction with a food web model provides a better understanding of the distribution and transformation of DPE in aquatic ecosystems. The model can be used in other aquatic environments, with their own system specific and chemical specific parameters. The model is expected to be useful for preliminary ecological risk assessment in order to predict exposure concentrations, internal body burdens and possible remediation targets.

**266 Measurement of Nanoparticles in Food and Biological Samples** R. Peters, RIKILT, Wageningen Univ, Residues & Contaminants; S. Weigel, H. Bouwmeester, RIKILT, Wageningen Univ. A variety of nanomaterials are being used in consumer products, in food packaging materials, in some food supplements and even some food products; e.g. titanium-, iron- and zinc oxides, silver and silica. As a consequence, diffusion into the environment and direct and indirect consumer exposure to nanoparticles is likely. Detection and characterization of nanoparticles in products, food, biological and environmental samples is an essential part of understanding the potential benefits as well as the potential risks of the application of nanoparticles. Silica is added to food items as an anti-caking agent known as E551. While this normally exists of micro-sized particles the material is a nano-formulated material with primary particle sizes in the nano-range. Hydrodynamic chromatography combined with ICPMS (HDC-ICPMS) is used to determine silica nanoparticles in food items and in models mimicking human digestion to determine the biological availability of nano-sized silica. Using HDC-ICPMS the presence of nano-sized silica in several food items could be shown and subsequent measurements in the biological model systems indicate that this nano-sized silica remains available after digestion. Single particle ICPMS (SP-ICPMS) is used as a screening tool for

nanoparticles in food and biological samples. In SP-ICPMS nanoparticles in the sample are introduced into the ICPMS plasma producing a plume of analyte ions resulting in a signal spike in the mass spectrometer. From this the particle size, size distribution and concentration can be calculated. The method was validated using the NIST gold reference materials 8011, 8012 and 8013 and tested for the determination of other nanoparticles such as silver, titanium-, iron- and cerium oxide, and silica. It is used in practice for the determination of gold, silver and titanium oxide nanoparticles in food supplements, in translocation experiments with cellular monolayers, and in biological samples from digestion model studies. In addition, the method was used to detect silver nanoparticles in the livers of rats that were exposed to silver nanoparticles through their food.

**267 Nanoeotoxicological Research Tools: Current State of Affairs and Future Needs** R. Tantra, National Physical Laboratory, Division of Analytical Sciences. Nanomaterial characterisation is integral to understanding the toxicological implications of such materials in the environment. The accuracy and reliability of data will be crucial if we are to understand the toxicity of nanomaterials and subsequently control the risk by monitoring key parameters linked to toxicity. Knowing what to measure and how to measure it are the main challenges. In this talk, we will be presenting an overview of progress in the use of various techniques, which are being used to obtain a comprehensive set of characterisation parameters under the PROSPEC LINK project. This project represents the UK's contribution to a recent OECD (Organisation for Economic Co-operation and Development) sponsorship programme for the testing of manufactured (CeO<sub>2</sub> and ZnO) nanomaterials, in relation to their potential risk of damage to the aquatic environment. We will discuss the various techniques employed (from routine based tools such as SEM, CPS disc centrifuge, Doppler Microelectrophoresis to more state-of-the art techniques such as TOF-SIMS) their advantageous and limitations in their applications in nanoeotoxicological research. Our findings have shown that although existing characterisation tools are of some use in a research setting, there is no doubt that new platforms and reference materials are very much required. In particular, for the purpose of classification and regulating nanomaterials, the development and validation of high-throughput platform is needed.

**268 Speciation Analysis of Silver Nanoparticles and Silver Ions in Environmental Waters and Antibacterial Products** J. Liu, J. Chao, S. Yu, R. Liu, Z. Tan, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The rapid growth in the commercial use of silver nanoparticles (AgNPs) will inevitably increase silver exposure in the environment and the general population, and development of methods for analysis of AgNPs is needed. We developed a method for the separation, identification, characterization and determination of trace AgNPs in environmental waters. AgNPs were selectively concentrated from environmental water samples without disturbing their sizes and shapes by Triton X-114 (TX-114)-based cloud point extraction (CPE). The enrichment factor was 100 and the recoveries from various environmental samples were in the range of 57-116% at 0.1-146 µg/L spiked levels of AgNPs. The AgNPs pre-concentrated in the TX-114-rich phase were identified by TEM/SEM-EDS/UV-Vis spectrum, and quantified after microwave digestion by ICP-MS with a detection limit of 0.006 µg/L. This proposed method can be adopted to track of AgNPs in environment. The TX-114-based CPE was also used as an efficient separation approach for the speciation analysis of AgNPs and Ag<sup>+</sup> in antibacterial products and environmental waters. AgNPs were quantified by determining the Ag content in the TX-114-rich phase with ICP-MS after digestion, the total silver content was determined by ICP-MS after digestion, and the total Ag<sup>+</sup> concentration was obtained by subtracting the AgNP content from the total silver content. The limits of quantification (S/N = 10) were 0.4 µg/kg for AgNPs, 3 µg/kg for total Ag<sup>+</sup>, and 0.2 µg/kg for total silver, respectively. This proposed method is applicable for studying the transformation of AgNPs in environment and the toxic effects of AgNPs and Ag<sup>+</sup>. To elucidate the toxic roles of the freely dissolved Ag<sup>+</sup> and the AgNP-adsorbed Ag<sup>+</sup>, hollow fiber supported liquid membrane (HFSLM) extraction was coupled with ICP-MS for speciation analysis of Ag<sup>+</sup>. Two extraction modes of HFSLM, the negligible depletion extraction and the full depletion extraction, were coupled to ICP-MS for analyzing the freely dissolved Ag<sup>+</sup> and the total Ag<sup>+</sup> content, respectively. The AgNP-adsorbed Ag<sup>+</sup> was obtained by the difference between the total and the freely dissolved Ag<sup>+</sup> content. The recoveries of total Ag<sup>+</sup> in AgNPs dispersions ranged from 86% to 98% at spiking levels of 10 ppb and 20 ppb Ag<sup>+</sup>. The

detection limits of freely dissolved Ag<sup>+</sup> and total Ag<sup>+</sup> were 0.9 ppb and 1.5 ppb, respectively.

## 269 Nanomaterial Release from Products is not Related to Hazard

**Data: Methods and Data Lacking** S. Froggett, (consultant) Center for Risk Science Innovation and Application, ILSI Research Foundation; R.A. Canady, Center for Risk Science Innovation and Application, ILSI Research Foundation. Advances in incorporation of engineered nanomaterials (ENM) into composites have occurred in tandem with the identification of possible hazards of the pristine ENM prior to compositing. However, review of research of release of ENM from nano-composites conducted for the NanoRelease project ([www.ilsi.org/ResearchFoundation/Pages/NanoRelease1.aspx](http://www.ilsi.org/ResearchFoundation/Pages/NanoRelease1.aspx)) shows little attention is being paid to ENM release from the composites. Consequently, it is not clear whether potential risks of that material are informed by current ENM hazard research. A crucial finding of the review is that our understanding of the risks associated with uses of nanomaterials will continue to be insufficient until substantial progress is made on methods to measure ENM release from uses of ENM. In the available reports, consumer products containing nano-silver, nano-titania and carbon nanotubes have been investigated under several exposure scenarios, including, weathering, abrasion, UV and washing. The data show three possible release scenarios: nanoscale particles of some kind (almost always, similar to materials without added ENM), nano-scale particles with some carrier matrix and some ENM (often), and finally, ENM alone (rare). This review of the nanomaterial release literature illustrates a data gap relevant to the field of environmental risk assessment. Perhaps most critical, our review suggests that fate and transport modeling, exposure assessment, and risk assessment frameworks in general for ENM uses should not be based on pristine engineered nanoparticles, but rather should be based on the released composites of ENM and the carrier matrix.

## 270 Comparison of Two Separation Techniques, Asymmetrical Field Flow Fractionation and Hydrodynamic Chromatography, for Mixtures of Gold Nanoparticles

E. Gray, Colorado School of Mines, Environmental Science and Engineering; T. Bruton, Arizona State Univ; p. westerhoff, Arizona State Univ, Arizona State Univ, School of Sustainable Engineering and The Built Environment; R. Halden, R. Halden, Arizona State Univ; J. Ranville, Colorado School of Mines, Chemistry and Geochemistry, Colorado School of Mines; C. Higgins, Colorado School of Mines, Environmental Science and Engineering; P. Herckes, Arizona State Univ. In recent years concern has been raised over the potential for environmental release of engineered nanoparticles (ENPs) as a result of increased ENP use and production. Robust methods to detect and characterize these ENPs in environmental samples are still being developed. This paper compares two techniques currently applied to ENPs; hydrodynamic chromatography (HDC) and asymmetrical flow field flow fractionation (AF4), both coupled with inductively coupled plasma mass spectrometry (ICP-MS). The goal of this study was to determine and compare the detection limit, resolution, and recovery obtained when separating mixtures of gold particles using common flow parameters, using each technique. The limit of quantification for both techniques is approximately 5 mg/L and the limit of detection is an order of magnitude lower at 500 µg/L. AF4 is capable of separating mixtures of 5, 20, 50 and 100 nm gold ENPs with greater resolution than HDC. These resolution differences are most pronounced at the lower size range of 5 to 20 nm. Sample recovery ranged from 67 to 81 percent for HDC while AF4 recovery values range from 31 to 68 percent. The elevated sample loss observed using AF4 results from both reversible and irreversible particle attachment to the semipermeable membrane used in the AF4 channel.

## 271 Novel Approaches for the Detection of Single Walled Carbon Nanotubes in Environmental Matrices

P. Schierz, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Pratt School of Engineering; A.N. Parks, P. Ferguson, Duke Univ. Single-walled carbon nanotubes (SWNT) are considered a new class of emerging environmental contaminants. Fate and toxicity studies rely heavily on reliable analytical methods to detect SWNT in environmental matrices at environmentally relevant concentrations [ppb-level]. NIRF spectroscopy has advanced as a highly selective and information-rich technique for sensitive detection and structural characterization of SWNT materials. We have demonstrated the capabilities of this technique through the quantitative analysis of SWNT in sediment and biotic matrices at environmentally relevant concentrations. The analytical power of NIRF allows for the structural characterization of SWNT samples

before and after toxicity, bioavailability and fate studies. Furthermore, for an assessment of the environmental fate of SWNT such as transport behaviour and bioavailability, structural information including shape, length distribution and agglomeration state are essential. Our approach to address these analytical challenges is the use of asymmetric field flow fractionation AF4 as a separation method prior to NIRF spectroscopic analysis to determine SWNT length distribution and to reduce matrix complexity by separation of NOM and SWNT. Metal catalyst residues (e.g., Co, Mo, Ni, Fe, etc.) are known to be associated with SWNT as a byproduct of the manufacturing process. We have examined the potential of AF4 coupled with inductively coupled plasma-mass spectrometry (AF4-ICP-MS) to trace CoMoCat SWNT in environmental samples from the presence of residual Co and Mo metal catalysts. The use of AF4-NIRF and AF4-ICP-MS in concert will facilitate a more thorough understanding of the environmental fate, transport, and behavior of SWNT.

## 272 Screening Waters Impacted by Municipal Wastewater for Metal-bearing Nanoparticles by Single Particle-Inductively Coupled Plasma Mass Spectrometry

E.M. Heithmar, US Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, USEPA, Research Chemist; S.A. Pergantis, Univ of Crete, Chemistry; E.M. Siska, Student Services Contractor to EPA. Metal – and metal oxide- based engineered nanomaterials are being increasingly used in consumer products in either intentionally or incidentally dispersible forms, and their release into municipal wastewater streams is inevitable. In order to obtain an estimate of their current occurrence and an upper-bound of their concentration in waters impacted by municipal wastewater, a rapid method for screening these waters is required. We have previously presented a preliminary application of single particle-inductively coupled plasma mass spectrometry (SP-ICPMS) for the screening-level analysis of surface water for metal-bearing nanoparticles. In this study, we apply SP-ICPMS to analysis of water significantly impacted by municipal wastewater. The increased continuous background and matrix effects associated with the more complex matrix and methods for mitigating these will be discussed. The benefits of recently introduced instrumentation on the analysis will be demonstrated. In addition to targeted determination of silver-, titanium-, and cerium-bearing nanoparticles, a novel approach to rapidly screen for a range of metals will be presented. Results for several water samples taken immediately downstream from municipal wastewater treatment plants in the US will be presented.

## 273 Comparison of Nanoparticle Characterisation Methods Using Specifically Tailored Multimodal Particle Suspensions

A. Jamting, National Measurement Institute, Nanometrology Section; V.A. Coleman, H.J. Catchpoole, M. Roy, J. Herrmann, National Measurement Institute. Understanding the impact of engineered and naturally occurring nanoparticles on environmental and biological systems is of critical importance, particularly when assessing risks. In order to evaluate the impact, nanoparticle systems need to be fully characterised to correlate physico-chemical properties with observed effects. It is therefore essential that the characterisation instrumentation is rigorously understood in terms of detection sensitivity, measurement range and validity. The National Measurement Institute Australia has established a comprehensive nanoparticle characterisation laboratory and is developing expertise in a range of characterisation methods for nanomaterials. One of the complexities of this area of investigation is that different instruments measure different physical properties or measurands to derive particle size. Here we present results from a range of measurements on multimodal suspensions of well characterised materials to highlight the benefits and limitations of different particle sizing instrumentation. The techniques investigated include dynamic light scattering, differential centrifugal sedimentation, transmission electron microscopy, asymmetric flow field-flow fractionation coupled with static and dynamic light scattering detectors, nanoparticle tracking analysis and a recently developed instrument based on single particle measurements using a micro-channel resonator, providing ultra high-resolution mass sensing.

## 274 Cumulative Impacts of Mountaintop Mining on an Appalachian Watershed

T. Lindberg, R. Di Giulio, Duke Univ, Nicholas School of the Environment; E. Bernhardt, Duke Univ, Biology. Surface mining is the dominant driver of land cover and land use change in Northern and Central Appalachia. Some watersheds in West Virginia have more than 25% of their area covered by surface mine permits. Studies show that at 10% there is a

decline in biodiversity and water quality. Our study system on the Upper Mud River in West Virginia, USA has been extensively mined since the 1970's. From its headwaters to a point 14km downstream, 49% of the 56sq. km watershed has been impacted by surface mining. During our study we found average stream conductivity to be 10 to 15 times greater than typical unmined Appalachian streams. During four monthly surveys we sampled the stream 52 times at 15 sites and found that average stream conductivity increased from 156  $\mu\text{S cm}^{-1}$  upstream of mining activity to 1525  $\mu\text{S cm}^{-1}$  downstream of the last impacted tributary. Our synoptic approach to sampling the Upper Mud River was novel in that we were able to capture water chemistry data from the headwaters above all current and historical surface mining, within each affected tributary and then from points upstream and downstream of each confluence. At this scale we were able to show conclusively the cumulative impacts on mainstream water quality due to multiple inputs from watersheds that receive surface mine effluents. To further quantify the connection between surface mining and water quality we utilized spatial analysis to measure the areal upstream proportion of the watershed that was surface mined at 15 sampling points on the mainstem and then correlated that to the measured concentrations of mining related pollutants and conductivity. We found that increasing concentrations of sulfate, selenium, magnesium, calcium, and others were significantly correlated ( $R^2 > 0.85$ ,  $p < 0.0001$ ) to the amount of upstream mining disturbance. Median conductivities had a correlation of 0.96 meaning almost all of the change in conductivity from upstream to downstream sites could be explained by the amount of upstream surface mine activity in the watershed. We also provide evidence that although reclamation of these mines may provide some decrease in the mobilization of nitrogen and selenium; it appears that dissolution of weathering related minerals that contribute to high conductivity in the receiving stream remain high decades after reclamation.

**275 Longitudinal Effects of Coal Mining/Valley Fills on Benthic Invertebrate Communities of Streams in Southern West Virginia** S.P. Canton, GEI Consultants, Inc., Ecological Division; J.S. Lynch, GEI Consultants, Inc.; R.W. Gensemer, GEI Consultants, Inc., Ecological Division; G.D. DeJong, C.F. Wolf, GEI Consultants Inc. We sampled the benthic macroinvertebrate populations of 12 streams in southern West Virginia, including nine streams with valley fills present in the upper reaches and three reference streams with no mining impacts. The proposed ecological impacts of coal mining/valley fill (CM/VF) operations on benthic invertebrate communities have been linked primarily to elevated concentrations of total dissolved solids, but spatial variability in water quality and benthic communities is poorly understood in these systems. The goal of this study was to determine the nature and extent of potential impacts that exist from the valley fill and associated impoundment and the downstream extent of those impacts. Parameters analyzed included density, number of taxa, number of EPT taxa, and relative abundance of mayflies, concurrent with matching water quality parameters (i.e., temperature, conductivity, calcium, potassium, sodium, magnesium, carbonate, bicarbonate, sulfate, chloride, pH, hardness, nitrate-N, and selected metals), habitat, periphyton, and detrital parameters. Longitudinal differences between sites on the same stream and between CM/VF and reference streams were observed, with some streams showing improvement in metric values in a downstream direction. Most invertebrate parameters were significantly different between CM/VF and reference streams at the most upstream site but were not significantly different at the most downstream site. Correlations between invertebrate metrics and water quality and habitat parameters were observed. Analysis of the pooled water chemistry data indicated that most of the differences observed between CM/VF and reference sites were statistically significant ( $p \leq 0.01$ ). CM/VF sites had significantly higher median values than reference sites for bicarbonate, calcium, chloride, magnesium, nitrate, potassium, selenium, sodium, sulfate, conductivity, hardness, pH, and temperature. We conclude that invertebrate assemblages in streams below valley fills did, indeed, differ from those in reference streams in some respects, with invertebrates often being abundant in both types of streams but having fewer taxa and EPT taxa represented in CM/VF streams. Overall, it appears the composition of the invertebrate assemblages observed in these streams varies as a result of a combination of ionic chemistry, soil characteristics related to iron and aluminum, substrate characteristics, and channel features related to erosion.

**276 Do Water Quality and Habitat Conditions Affect Macroinvertebrate Communities in Headwater Streams in Southern West Virginia?** S.P. Canton, GEI Consultants, Inc., Ecological Division; G.D. DeJong,

R.W. Gensemer, GEI Consultants, Inc. We described the benthic invertebrate communities inhabiting the extreme upper reaches of headwater streams in the coal mining region of southern West Virginia. These headwaters are representative of ephemeral or intermittent streams where mountaintop coal mining/valley fill (CM/VF) operations occur. The proposed ecological impacts of CM/VF operations on benthic invertebrate communities have been linked mostly to elevated concentrations of total dissolved solids, but direct loss of headwaters communities is also of concern. Most ecological studies conducted in the extreme headwaters of freshwater streams focus primarily on terrestrial/aquatic linkages and not the composition of the aquatic communities. Therefore, we sampled 12 headwater streams in three different protected natural areas of southern West Virginia to evaluate the natural variability of their benthic invertebrate communities. A diverse benthic invertebrate community was found, consisting of at least 129 benthic invertebrate taxa. Variability analyses revealed low levels of similarity between the invertebrate communities in headwaters streams. Benthic habitat was similar among sites, with the same kinds of habitat features occurring at most sites. Correlation analyses of water chemistry parameters (conductivity, calcium, potassium, sodium, magnesium, carbonate, bicarbonate, sulfate, chloride, pH, hardness, nitrate-N, and selected metals) with benthic invertebrate metrics (number of taxa, number of EPT taxa, percent EPT taxa, and Ephemeroptera abundance) showed that water chemistry parameters were not strong predictors of the benthic invertebrate metrics. The strongest relationship was a negative correlation between potassium concentration and the number of EPT taxa. Only two correlations between water chemistry and invertebrate community parameters were statistically significant ( $p < 0.050$ ), both involving potassium concentrations. There were no significant correlations between conductivity and any benthic invertebrate metric. pH was low ( $< 6.0$ ) at some sites where EPT taxa were not encountered as frequently, but this was not a consistent pattern. Our study suggests that the taxa encountered and levels of dissimilarity between the invertebrate communities in headwaters streams with otherwise similar habitat and water quality are consistent with the hypothesis that headwaters communities are established opportunistically from a larger regional pool of organisms.

**277 In Situ Quantification of Major Ions to Improve Detection/Remediation of Threats to Environmental Health** A. Mueller, H. Hemond, Massachusetts Institute of Technology, Dept of Civil and Environmental Engineering. It is well known that the quantity and balance of major ions has profound effects on ecosystem structure and health and can drive changes in natural floral and faunal species. Unfortunately, ecological studies are often forced to rely exclusively on indicator measurements, such as pH and electrical conductivity, when completing in situ assessments due to the high cost of traditional grab sampling campaigns. This generally leads to availability of only incomplete and low-resolution data which may not provide the information necessary to diagnose or remediate threats to specific ecosystems. One such historical example was identification of the causes of stream acidification: while sulfate deposition was initially suspected, further research pointed to substantial contributions from nitrogen species as well. In contrast, data covering the full spectrum of dissolved ionic species and available in real-time would provide researchers the ability to lead high-resolution, adaptive sampling campaigns from the start, resulting in more precise identification of problem regions and better targeted grab sampling should high accuracy lab analysis be required. To address this gap, we have undertaken development of an in situ instrument for real-time identification of dissolved ion species, initially targeting both the dominant ionic species and those frequently involved in eutrophication processes, specifically:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ , and  $\text{H}^+$ . This instrument relies primarily upon ion selective electrode technology, coupled with novel signal processing algorithms that allow mixed responses to be successfully decoupled. Relevant environmental parameters, e.g., temperature and electrical conductivity, are measured simultaneously and provide additional information to improve the accuracy of the signal processing results. An initial test of this combined hardware/software technique has been undertaken using data provided by the US Geological Survey, collected over the past 50 years using traditional sampling techniques. Response of instrument hardware to similar samples allows simulation of sensor responses to all points in this 50-year data set. Samples are then run through the software algorithm with results analyzed for both accuracy and precision over a wide range of ionic concentrations.



**278 Total Dissolved Solids Concentrations of Tecolote Creek, San Diego, CA; Effects on Sentinel and Native Species**

B.J. Mastin, Weston Solutions, Inc., Natural Resources; S. Gruber, Weston Solutions, Inc., Weston Solutions; W. Isham, Weston Solutions, Inc., Natural Resources. Identification and measurement of ionic strength as a causative agent of toxicity is difficult due to site-specific, species-specific, and ion-specific responses by freshwater biota. Concentration and composition of ionic strength are influenced by the localized topography and geology of the drainage, atmospheric deposition, changes in land use, storm water management, irrigation, agricultural activities, industrial effluents, salting of roads, and other anthropogenic activities which can alter the associated hydrology of the watershed. For example, researchers have documented that if untreated, increased TDS concentrations of oilfield produced waters, hydraulic frack fluids, and mine drainages were the causative agent of toxicity to downstream biota and a primary source of benthic community impairment. Ionic strength will increasingly influence biotic diversity in sensitive arid and semi-arid regions as climate patterns continue to shift our regional water balances. Recently, several states (e.g., Florida and West Virginia) have adopted water quality criteria which address the importance of monitoring ionic strength for the protection of freshwater biota. The objective of these experiments was to measure potential effects of total dissolved solids (TDS) concentrations of Tecolote Creek (an urbanized watershed in San Diego, CA) during dry weather on both native and sentinel laboratory species that represent the macroinvertebrate diversity and sensitivity residing within the Tecolote Creek Watershed. Serial dilutions (i.e., six concentrations) were prepared with high conductivity water (> 5,000 to 11,000  $\mu\text{S}/\text{cm}$ ) collected from Tecolote Creek tributaries and diluted with water from an un-urbanized reference creek (approximately 300  $\mu\text{S}/\text{cm}$ ). Toxicity bioassays with sentinel laboratory species included *Ceriodaphnia dubia* (48-h survival and 7-d reproduction), *Hyalella azteca* (48-h survival), *Chironomus tentans* (48-h survival), *Rhaphidocelis subcapitata* (96-h growth), and an *Isonychia* spp. (48-h survival) and native test organisms including both an Ephemeroptera (mayfly) and Plecoptera (i.e., stonefly) species (48-h survival). *C. dubia* reproduction was the most sensitive endpoint to increased ionic strength compared to *H. azteca* and *C. tentans* survival which was not observed to be affected in this high TDS water. Preliminary results of water chemistry and benthic community assessment will also be discussed as part of this presentation.

**279 Potential Impacts from Short-duration Exposures to Storm Water Runoff on the Rocky Intertidal Community of a Marine Preserve**

S. Gruber, Weston Solutions, Inc., Weston Solutions; D. McCoy, Weston Solutions, Inc.; B.J. Mastin, Weston Solutions, Inc., Natural Resources; R. Kolb, City of San Diego. As part of a larger study for the City of San Diego, Weston Solutions assessed the impacts of storm water on the rocky intertidal biological community in the La Jolla Area of Special Biological Significance (ASBS), a designated marine preserve. The assessment used a triad approach: water chemistry, toxicity to native marine species, and algal and biological surveys. Conditions during a storm event at the La Jolla ASBS were compared to a coastal reference site (Cabrillo National Monument), which is not influenced directly by storm water runoff. Water samples were collected for chemical and toxicological analyses at the La Jolla site over the course of a storm event (i.e., pollutograph) directly from a storm drain outfall as well as from the ocean receiving waters in the ASBS rocky intertidal habitat. Storm water samples were used in the laboratory to mimic the exposure to storm water that marine test species (e.g., abalone, sea urchins, diatoms, bivalves, and mysid shrimp) would experience over a tidal cycle (i.e., 6-h storm water exposure followed by a seawater flush for the duration of the test). Chemistry results showed that, during low tide, concentrations of pyrethroid pesticides and some metals at the La Jolla site were similar to those in storm water runoff and greater than LC50s of native marine organisms. Salinity at the La Jolla site was less than 3 ppt in the receiving waters during low tide, but never fell below 32 ppt at the reference site during the same storm event. Toxicity results indicate that short-term exposure (6 hours) to storm water runoff is toxic to marine organisms and exposure to fresh water during a storm may have more of an impact than exposure to pesticides and metals associated with the storm water outfall. Biological surveys showed distinct differences between the La Jolla ASBS and reference sites, possibly reflecting the long-term exposure to storm water runoff over several decades. The results have important implications for managing storm water discharges to ocean receiving waters and the ASBS with respect to new regulations designed to protect the beneficial uses of this important coastal habitat.

**280 Short-term and Long-term Exposure Impacts of Diluted Desalination Brine Discharge to Various Biological Receptors**

S. Bodensteiner, Weston Solutions, Inc., Weston Solutions. Available disposal options for the high-salinity sidestream generated during the desalination process (i.e., brine) is a key limiting factor with construction of seawater desalination plants. If there is not a nearby or co-located wastewater treatment plant generating fresh water effluent available to blend with a high salinity brine stream, then a desalination plant must rely on ambient seawater to dilute the brine to an ecologically innocuous salinity level. This is commonly accomplished by blending brine with either receiving water intake or cooling water discharge from a co-located power plant. Seawater reverse osmosis desalination plants are typically designed for recovery of 45% to 55% as fresh water, leaving a brine discharge salinity of approximately twice that of marine receiving waters. The operational costs for seawater desalination plants is therefore influenced by the volume of ambient seawater necessary to dilute the brine to a level that does not pose any significant threat to marine life within the discharge environment. Although it has been demonstrated that salinity levels up to 57 parts per thousand have not caused statistically significant changes to the overall function and quality of the aquatic environment in the discharge area, most desalination plants are restricted by discharge permit criteria that are based on toxicological impacts to single sensitive receptors. This study evaluates the impacts of varying brine discharge dilutions to critical life stages of several biological receptors in both short-term and long-term exposure scenarios. The suite of receptors was a mix of species accustomed to either hard surface or sandy bottom substrates in order to represent the two most common marine discharge environments. In addition to a comprehensive assessment of short and long-term exposure impacts to brine diluted with ambient seawater, the study also investigated potential effects of different ion ratios within the standard suite of Total Dissolved Solids (Ca, Mg, Na, Cl and  $\text{SO}_4$ ). This component was included to assess the differences in ecological impact between brine discharges diluted with ambient seawater and those diluted with effluent from wastewater treatment plants. In general, the occurrence of significant impacts was driven primarily by the type of life stage evaluated.

**281 Case Study of Effluent Discharged in Transition Zone of Freshwater and Estuaries**

W.L. Goodfellow, EA Engineering Science & Technology, Inc.; W. McCulloch, EA Engineering, Science & Technology, Inc., Dept of Ecotoxicology; M.K. Chanov, EA Engineering, Science and Technology, Inc., Dept of Ecotoxicology. Industrial wastewater containing total dissolved solids that are discharged to estuaries or rivers are often complicated by the regulatory classification of the ecosystem. In situations that are in this transition from freshwater to estuarine systems, the organisms that are used for the whole effluent toxicity assessment are typically not organisms that are found in this system and the WET test is not always an indicator of the effluent's risk to the environment. Through the presentation of this case study, this paper will present the potential impacts of a TDS discharge to a large river in an upper estuary and provide recommendations and observations for these effluent discharge situations.

**282 Linking Science and Policy to Inform Global Mercury Negotiations**

N.E. Selin, Massachusetts Institute of Technology, Engineering Systems Division. Global negotiations to address the risks posed by mercury are informed by scientific input of different forms, from peer-reviewed scientific papers to structured scientific assessments to informal science-policy dialogue. Recent insights from global-scale mercury modeling are highlighted in the context of ongoing negotiations towards an international, legally-binding mercury instrument. In particular, the role of historical versus present-day sources is highlighted, focusing on the long-term prospects for mercury exposure reductions. Additionally, the process by which science informs international mercury negotiations is examined and illustrated. Results from multiple iterations of a role-playing simulation focused on mercury are used to draw lessons about the uses and effectiveness of the provision of scientific information to international negotiators. Results are used to suggest potential avenues for scientific participation in the final two sessions of the global mercury negotiations.

**283 Climate Change as a Driver for Mercury Bioaccumulation in Aquatic Ecosystems: Implications for Mercury Emission Control Under a Changing Climate** F. Wang, Univ of Manitoba, Centre for Earth Observation Science, Dept of Environment and Geography. The stubbornly high and spatiotemporally variable concentrations of mercury (Hg) in Arctic

marine mammals have been a major subject of scientific research and policy debate. There is no doubt that Hg emission from anthropogenic activities in southern latitudes has played a major role in Hg contamination in Arctic marine ecosystems. What makes the Hg contamination phenomenon in the Arctic especially puzzling is, however, the apparent lack of an atmospheric-biological dose-response relationship in recent decades. Based on extensive research in the Arctic Ocean, we recently published a hypothesis that, under a rapidly changing climate, internal post-depositional processes within the system can overtake external emission sources in controlling Hg bioaccumulation in aquatic ecosystems. This hypothesis is further supported by new data from the Arctic Ocean and the Equatorial Pacific Ocean where climate-induced changes in the cryosphere and marine primary productivity, respectively, are shown to significantly modify the atmospheric Hg loadings. This shift in the paradigms in driving Hg bioaccumulation suggests that during a rapidly changing climate, emission control of Hg will be followed by long delays before ensuing reduction is seen in food-web Hg levels. The response lag makes it all the more urgent for the UNEP treaty to control further loading of Hg (and potentially other types of contaminants) into key environmental reservoirs. It also highlights the need of partnerships among scientists, policy-makers and local communities to develop short- and long-term adaptation and remediation strategies before the effectiveness of Hg emission control is realized.

**284 Global Mercury Trends and Local Ecosystem Recovery** L. Levin, Electric Power Research Institute, Air Toxics Health & Risk Assessment; K. Lohman, Environ Corp. As national steps are taken to reduce mercury emissions to the atmosphere, one issue remains unresolved: how much and how quickly will domestic ecosystems respond to reduced emissions from distant and nearby sources and the resulting changes in atmospheric deposition. Aquatic fish populations act as band-pass filters for mercury deposition and methylation over time, serving as interannual transfer systems for methylmercury mass within the system. Local and regional emission changes may be overlain by longer-range opposing trends in emissions and complex responses in water bodies and ecosystem receptors. Recent work by the HTAP (Hemispheric Transport of Air Pollutants) conference and other researchers shows how sensitive and with what rate local ecosystems may respond to global and local changes.

**285 Integrating Knowledge to Inform Mercury Policy: The IKIMP Initiative** J. Holmes, M.N. Gardner, T.A. Mather, D.M. Pyle, M.L. Witt, Univ of Oxford, Dept of Earth Sciences. It is essential that national and international initiatives to reduce the risks posed by mercury in the environment are informed by the best scientific knowledge of the sources of mercury in the environment and the options for reducing future human exposure to mercury. This paper will summarise the outputs of a project undertaken over the last three years at the Univ of Oxford to develop, integrate and present this knowledge to inform policy making at UK, European and global levels. Effective action to address anthropogenic emissions of mercury requires an understanding of the relative contributions of anthropogenic and natural sources, in particular from primary emissions to the environment such as crustal degassing. An updated national inventory has therefore been developed for the UK, identifying mercury sources and waste streams, and evaluating the adequacy of current monitoring arrangements. At a global level, an improved understanding of natural reservoirs of mercury, exchange fluxes between them, and the impact of climate change has been sought. Following evaluation of uncertainties in anthropogenic emissions of mercury, the project has focused on a review of mercury arising from the oil and gas industries. Policies are being developed nationally and internationally to withdraw mercury from the socio-economic system, requiring options to be developed for mercury storage and disposal. The current state of development of these storage/disposal technologies has been reviewed, and a decision framework has been developed which has informed discussions associated with the United Nations Environment Programme's initiative to develop a global instrument on mercury. A key aim of the project has been to enhance the availability of relevant information and understanding to policy processes at national and international levels. The paper will reflect on our experience of policy engagement over the last three years.

**286 Mercury Remediation in Kazakhstan: Exploring Interactions Between Nanoscale Zero-valent Iron and Sulfate-reducing Bacteria** A. Kajenthira, Harvard Univ, Harvard Kennedy School; I. Thompson, Oxford Univ, Dept of Engineering Science; A. Crossley, Univ of Oxford, Dept of

Materials; M.N. Gardner, Univ of Oxford, Dept of Earth Sciences. The ecological and human health risks posed by the volatility of mercury in the environment are undeniable. Although UNEP's internationally binding treaty on the use of mercury is anticipated in early 2013, the global legacy of mercury contamination has necessitated continued research into efficient and cost-effective remediation technologies. This study focused on the remediation of a groundwater mercury plume associated with the Khimprom Plant in Kazakhstan, and evaluated the potential of nanoscale zero-valent iron (nZVI) particles for the immobilization of Hg(II) in aqueous media. Although nZVI has shown substantial potential for the remediation of chlorinated organic solvents and heavy metals, its possible adverse effects on the bioavailability of other toxins or nutrients, as well as other indirect ecosystem effects, are largely unknown. More specifically, nZVI has been reported to have the potential for cell membrane disruption in *E. coli*, but its possible influence on obligate anaerobes – such as the sulfate-reducing bacteria that form the largest biological component of the global mercury cycle – remain unclear. Further, as the proliferation of sulfate-reducing bacteria can be highly sensitive to the bioavailability of iron, the application of nZVI could impact both mercury and iron concentrations within the environment. This study evaluates the impact of nZVI on the growth of a model sulfate-reducing bacterium: *Desulfovibrio desulfuricans* subsp. *desulfuricans* (DSM 6949), and measures aqueous iron concentrations following nZVI amendment with the goal of establishing the utility of nZVI as a Hg(II) remediation technology. It was found that nZVI had little or no significant detectable impact on bacterial growth kinetics when applied at concentrations of up to 100 mg/L. In addition, although aqueous Fe(II) ions resulted from the Hg(II)-nZVI sorption process, total Fe concentrations in solution returned to pre-amendment concentrations following the growth of DSM 6949 to maximum culture capacity. It was consequently hypothesized that aqueous Fe(II) ions were metabolized during the growth of DSM 6949. A complementary remediation approach for the Khimprom Plant that utilizes both nanoscale Fe(0) and sulfate-reducing bacteria could therefore enhance the overall potential for mercury immobilization while minimizing environmental impact.

**287 Methylmercury as a Brain Drainer** P. Grandjean, Univ of Southern Denmark, Environmental Medicine. The fetus and the child are particularly vulnerable to pollution. The fetus shares the mother's exposure and accumulated body burdens of pollutants, and many chemicals are transferred to the infant via human milk. In addition, during early life, cell differentiation and organ development must happen in a particular sequence and at particular times to create optimal functions of the mature organism. The brain is uniquely sensitive to toxicity during development, and adverse effects can have serious consequences, as we depend on the integrity of the complete organ for optimal functioning. Because there is only one chance to develop a brain, even doses that are safe for adults may, if occurring during early development, cause permanent IQ loss. Research on neurobehavioral deficits in humans relies on 'natural' experiments and faces many obstacles, in part because the best evidence requires prospective studies of birth cohorts lasting at least until school age. Because of the paucity of data, there has been a tendency to ignore risks of developmental neurotoxicity caused by methylmercury and other chemicals known to be hazardous to the human nervous system. However, during the last 10 years or so, methylmercury exposures previously thought to be safe have been reliably linked to adverse effects on brain development in children. As the children grow, the full impact of the toxicity is becoming apparent. We have now followed a Faroese birth cohort up to age 22 years and demonstrated that the methylmercury-associated effects do not disappear. So the neurodevelopmental delay must now be recognized as a permanent brain drain. The methylmercury experience is a reminder that research is unlikely to provide a sufficient basis for decision-making in regard to pollutants, especially since chemical substances are not systematically screened for neurotoxicity. As optimal brain function is crucial to each individual as well as to society, new and precautionary approaches are required both for research and for decision-making on chemical testing and control.

**288 US State Mercury Initiatives Demonstrate Need For Global Action To Reduce Mercury Pollution And Highlight Successful Strategies** C.M. Smith, Massachusetts Dept of Environmental Protection, Office of Research and Standards. Mercury (Hg) total maximum daily load (TMDL) analyses for New England (NE), NY, NJ and MN indicate that anthropogenic Hg inputs to freshwaters may need to be reduced up to 98% to achieve statewide targets for freshwater fish consumption. Due to air transport,

reductions in regional, national and global sources are needed. Many states are pursuing aggressive Hg reduction strategies and coordinate activities through the Environmental Council of States Quicksilver Caucus (QSC), a consortium of interstate organizations, and regional initiatives such as the New England Governors and Eastern Canadian Premiers Hg Action Plan (MAP). States have considerable flexibility to address environmental issues and have taken many steps to address Hg sources that exceed federal requirements. For example, the MAP, adopted in 1998, established a long-term goal of virtually eliminating Hg pollution sources in the region. The MAP uses an adaptive management approach and includes emission reduction milestones of 50% by 2003 and 75% by 2010 vs. a mid-1990s baseline (achieved); and, by 2010, use of amalgam separator pollution controls by 95% of dental offices (achieved by most jurisdictions). The MAP also includes pollution prevention, outreach and monitoring elements. A regional Hg Task Force (MTF) was established to coordinate MAP implementation, adjust elements of the MAP and report on progress to senior political and environmental leaders. Under the MAP stringent emission limits have been adopted for many sources including coal-fired power plants (e.g., MA regulations will require 95% control by 2012). NE states also adopted Hg products legislation requiring manufacturers to label Hg-added products and to: phase-out unnecessary uses; notify of ongoing sales; and, enhance recycling, of Hg-added products. Monitoring of Hg levels in freshwater fish in MA demonstrates improvement coincident with reduced local and regional emissions but levels remain too high. To address global sources that contribute significantly to Hg deposition the MTF and QSC are providing input to the USEPA and State Dept on the global Hg agreement negotiations. States have advocated for improved inventories of Hg releases and uses; improved monitoring; comprehensive multimedia planning that crosses traditional discipline and agency boundaries; adaptive management approaches; overarching stretch goals with measureable milestones; and reporting mechanisms to track progress.

**289 Wetlands – Implication on the Global Mercury Pollution** J. Canario, IPIMAR, Aquatic Environment Dept, INRB IP/IPIMAR, Aquatic Environment and Biodiversity; C. Vale, INRB IP/IPIMAR. The overall goal of the UNEP Global Mercury Partnership is to protect human health and the global environment from the release of mercury and its compounds by minimizing and, where feasible, ultimately eliminating global, anthropogenic mercury releases to air, water and land. Besides the decrease on mercury emissions in the northern hemisphere in the last decade, the amount of mercury circulating in the environment is still of great concern. This issue assumes a major problem in ecosystems, which due to their physical/chemical/biological characteristics, exchange mercury species mainly Hg<sup>0</sup> (mercury volatile) and MeHg (methylmercury) with the atmosphere and food web, respectively. Wetlands, particularly salt-marshes, are among these key ecosystems where these Hg transformations and Hg escape occur. Mercury studies in Portuguese salt-marshes showed that salt-marsh plants and the surrounding rhizosphere environment are important areas of mercury retention and methylation. Levels of up to 30% of MeHg in salt-marsh sediments contrasting to the 0.01% of MeHg in non-vegetated ones and higher concentrations of both Hg and MeHg in root biomass poses a serious debate about whether salt-marshes are efficient for Hg phytoremediation. Additional studies in Portuguese salt-marsh plants in contaminated areas also showed that macrophytes are important sources of Hg<sup>0</sup> to the atmosphere. Mercury vegetation-air fluxes measured in two salt-marsh plant species showed Hg<sup>0</sup> emissions during three consecutive days, indicating that these ecosystems can act also as an important natural mercury source. The export nature of these ecosystems for mercury has been shown. These findings have serious implications on mercury pollution especially concerning mercury fate and speciation in wetland contaminated areas. Based on our work we believe that the definition of a global mercury policy must take into account wetland areas which occupied up to 9 million km<sup>2</sup> of earth surface therefore incorporating these ecosystems in the UNEP monitoring and assessment mercury programs. Two types of measures should be envisaged. Encourage better understanding of mercury transformation and fluxes in key areas of the world, namely wetlands and areas where mercury is used in industrial processes. Improve technologies to reduce the mercury emissions and to sequester mercury in inorganic forms.

**290 Terrestrial Ecological Risk Assessment to Support RCRA Facility Management Decisions at the Hanford Nuclear Reservation** L. Williams, Integral Consulting Inc.; B. Tiller, Environmental Assessment Services; M.

Connelly, Washington River Protection Solutions. The Inner Area of the Hanford Nuclear Reservation's Central Plateau is a 10-square mile mosaic of industrialized areas and highly fragmented and often disturbed sagebrush-steppe habitat. The Inner Area has been used for nuclear fuel processing and waste management and waste disposal activities, including several tank farms that collectively had received 53 million gallons of radioactive waste. Past spills and leakages at the tank farms are suspected of releasing up to 1 million gallons of mixed radioactive and non-radioactive materials to surface and subsurface soils. Waste Management Area C is an 8.5-acre tank farm that is undergoing a RCRA facility investigation and cleanup pursuant to closure within the next few years. An ecological risk assessment is being undertaken for Waste Management Area C to evaluate threats to terrestrial habitat that may be associated with past releases of chemicals and radionuclides under a no-action cleanup alternative. The ecological risk assessment uses a site and receptor-specific approach that integrated requirements in EPA, DOE, and Washington State regulations or guidance. Plant, invertebrate and wildlife species that are indigenous to the Columbia Plateau were selected as receptors of interest. Risk will initially be evaluated by comparison of ecotoxicity thresholds with contaminant concentrations in soils. A secondary line of evidence based on tissue chemistry for small mammals collected in the vicinity of the tank farm will be used to assess risk to higher trophic level predators. The terrestrial ecological risk assessment approach will also be used to help define site-specific cleanup goals, to facilitate selection of corrective action alternatives and to assist in evaluating post-closure conditions associated with an engineered soil cover.

**291 Evaluation of Biointrusion in Support of Remedial Planning for the Central Plateau of the US Dept of Energy Hanford Site** B. Sample, Ecological Risk Inc.; P. Seeley, Cenibark International, Inc.; J. Lowe, CH2M HILL Plateau Remediation Company; M. Markin, CH2M Hill. Remediation of the Central Plateau of the Hanford Site requires definition of the remedial depth (i.e., the point of compliance). Under the State of Washington Model Toxic Control Act (MTCA), the point of compliance for terrestrial ecological considerations can be adjusted based on site specific conditions, the assumed biologically active zone (6 ft) or at the standard point of compliance assuming no institutional controls (15 ft). This study was undertaken to identify and bound the biologically active soil zone as an appropriate point of compliance. Literature on animal burrowing and plant rooting depths was identified. Considerable primary data are available for depths to which ants, mammals, and plants may exploit the surface soil column at the Hanford Site and other comparable locations. The maximum depth observed for harvester ants (*Pogonomyrmex* spp.) was 270 cm (8.9 ft), with only trivial excavation below 244 cm (8 ft). Badgers (*Taxidea taxus*) are the deepest burrowing mammal at the Hanford Site, with maximum burrow depths of 230 cm (7.6 ft); all other mammals did not burrow below 122 cm (4 ft). Shrubs are the deepest rooting plants with rooting depths to 300 cm (9.8 ft) for antelope bitterbrush (*Purshia tridentata*). Two of the most abundant shrub species did not have roots deeper than 250 cm (8.2 ft). The deepest rooted forb had a maximum root depth of 240 cm (7.9 ft). All other forbs and grasses had rooting depths of 200 cm (6.6 ft) or less. Spatial distribution and density of roots over depths were also evaluated. These data indicate that the biologically active soil zone in the Central Plateau does not exceed 300 cm (9.8 ft), the maximum rooting depth for the deepest rooting plant. The maximum depth at which most other plant and animal species occur is substantially shallower. No mammals burrow to depths greater than 230 cm (7.6 ft). Although the maximum excavation by harvester ants is 270 cm (8.9 ft), trivial volume of soil is excavated from depths below 150 cm (~5 ft). Most root biomass (>50-80%) is concentrated in the top 100 cm (3.3 ft), and at the maximum depth, only trace root biomass is present. Based on these data, a site-specific depth of 305 cm (10 ft) is proposed for the Central Plateau at Hanford Site as the depth in soil below which it may be reasonably expected that contaminants do not pose a threat to terrestrial plants or animals.

**292 Risk Based Soil Concentrations for the Protection of Wildlife at the US Dept of Energy Hanford Site** C. McCarthy, CH2M Hill, Environmental Services; B. Sample, Ecological Risk, Inc.; J. Lowe, CH2M HILL Plateau Remediation Company; M. Markin, D. Wright, H. Awata, CH2M Hill. Risk-based soil concentrations for wildlife were developed as part of a tiered framework for derivation and application of values to support risk management for the US Dept of Energy Hanford Site. Generic values were initially identified from published sources (EPA, MTCA, ORNL,



DOE) that correspond to the no-effect based thresholds generally used in screening-level ecological risk assessments (SLERAs) with applicability across North America or the State of Washington. Tier 1 values were derived, still as literature-based values, by employing the exposure dose models used in the EPA's EcoSSLs and DOE's A Graded Approach for Evaluating Radiation Doses to Aquatic and Terrestrial Biota but specifically for bird and mammal species present at the Hanford Site. Tier 1 values generally correspond to the effect thresholds that would be employed in SLERAs, Step 3b of EPA's Risk Assessment Guidance for Superfund, or Site-specific Terrestrial Ecological Evaluations under MTCA. Tier 1 values are intended to differentiate between COPECs, receptors, and locations for which a conclusion of no unacceptable risk can be made and those for which risk may exist but current data are insufficient to support a definitive conclusion. Tier 2 values were also developed for non-radionuclides by updating the exposure assumptions of the Tier 1 models through integration of available Hanford specific bioaccumulation data with that from existing data sets. Data for soil paired with plants, terrestrial arthropods, and small mammals from the data sets that supported existing bioaccumulation models (i.e., those from OSWER Directive 9285.7-55 used to develop the EcoSSLs) or from other published sources were combined with Hanford Site data to develop a set of partially site specific bioaccumulation models. For estimating exposure from consumption of invertebrates, only arthropod tissues were included to be more representative of the terrestrial environments found at the Hanford site where insects and other arthropods (e.g., spiders, etc.) are more likely to be consumed. Use of the Tier 2 values is intended to improve risk management decisions through the reduction of uncertainty associated with assumptions in the development of Tier 1 values. Tier 2 values correspond to effect thresholds to be used in a baseline risk assessment or as preliminary remediation goals (PRGs). Tier 3 values can be developed for specific localized application but have not been developed to date.

**293 Statistical Methods to Evaluate Soil Bioassay Results from Heterogeneous Soils and Multiple Collection Events** W. Swanson, R. Ryti, Neptune and Company, Inc.; D. Jacques, Washington Closure Hanford. Among the lines of evidence evaluated in ecological risk assessments, bioassays performed with site-specific soils can be one of the most relevant and diagnostic of the potential for adverse effects on specific receptors. However, the results of the bioassays vary as a result of laboratory conditions (moisture, light, test organism, etc) as well as various confounding factors related to soil properties and characteristics. This paper reviews soil bioassays performed to support ecological risk assessment of upland and riparian habitat in the River Corridor portion of the Hanford Site in Washington State. Seedling germination tests using a local native species, Sandberg's Bluegrass (*Poa sandbergii*), were conducted on surface-soil samples collected from over 50 study sites in the River Corridor. The soil samples were collected from 2005 to 2008 from a variety of study sites having a wide range of contamination levels and soil characteristics. In total there were 362 different bioassay tests evaluated for over 70 contaminants and confounding factors. Statistical methods were used to evaluate overall bioassay trends, the impact of confounding factors, and draw conclusions regarding adverse effect levels of contaminants. Results for the seedling germination tests are presented to illustrate these statistical methods and the challenges related to interpreting results from this type of test.

**294 Approach for Development of Plant and Invertebrate Preliminary Remedial Goals for the US Dept of Energy Hanford Site** B. Sample, Ecological Risk, Inc.; C. McCarthy, CH2M Hill, Environmental Services; A. Aly, Interra; J. Hansen, United States Dept of Energy. Available literature-based soil screening values may not reflect Hanford Site specific conditions that affect analyte specific exposure/response relationships for plants and invertebrates. Use of these screening levels to guide remedial decision-making introduces uncertainty that warrants further study. A site-specific bioassay program, using Sandberg bluegrass (*Poa secunda*) 14 day post germination and 28 day growth bioassays and springtail (*Folsomia candida*) 28-day reproduction and survival bioassays was conducted to address this uncertainty. Bioassay results and paired chemistry data were collected for 70 samples (11 field controls and 59 target locations) representing a range of concentrations of selected contaminants. A step-wise approach for evaluating these data and deriving site-specific effect thresholds was employed. First, the distribution of analytical chemistry was compared to existing screening benchmarks and Hanford background concentrations to a) ensure field control samples were less than background and b) identify analytes that may have an association

with effects. Second, specific samples were identified as toxic using statistical analysis (parametric and non-parametric ANOVA) to identify differences in responses among laboratory controls, field controls, and target samples and through the development of a 'reference envelope' based on background concentrations. Third, chemistry data were integrated with bioassay results. Correlation analyses were performed to identify analytes most associated with observed responses. Site-specific NOECs (highest concentration at which no effects were observed) and Apparent Effect Thresholds (lowest concentration at which effects were observed in all samples) were developed using the ANOVA and Reference Envelope results. Fourth, regression analyses (linear and nonlinear) were performed to quantify dose-response relationships and to identify 20 and 50 percent effects levels. Finally, principal components analysis was conducted to identify sets of analytes associated with observed bioassay response. The results of these analyses will be used to establish preliminary remediation goals (PRGs) that will have applicability to soils within terrestrial environments across the Hanford Site, which include both upland and riparian habitats.

**295 The Hanford Site: Ecological Risk Evaluation of the Columbia River** J.E. Robinson, Woodard & Curran Inc.; L.C. Hulstrom, Washington Closure Hanford. As part of the on-going cleanup of DOE's Hanford Site in Washington State, approximately 100 miles of the Columbia River adjacent to and downriver of the Hanford Site is being investigated and evaluated in accordance with the RI/FS process of Superfund. Although not technically part of the Hanford Site, the Columbia River lies adjacent to the facility and was a source of cooling water for nuclear reactors during the decades when the Site was operating. This presentation describes the process used to conduct a screening level ecological risk assessment of the main channel of the Columbia River. The study was based on both historical data and additional surface water, porewater, sediment, island soil, and fish tissue data collected during the recent remedial investigation. The fish sampling program included the collection of composite samples of fish tissue (fillets, kidney, liver, and carcass) from six species of resident fish. Statistical evaluation of sediment and surface water data was used to divide the River into four Sub-Areas, which were evaluated independently. Contaminants of potential ecological concern (COPECs) were selected statistically and evaluated against no-observed-effect-level (NOEC) benchmarks. Compounds exceeding NOECs were further evaluated as part of a Refinement that considered a variety of additional factors, including LOEC values and the number, magnitude, location, and date of LOEC exceedances. Potential effects on fish were evaluated by histopathological examinations, plus comparisons of fish weight, length, condition factors, and hepatosomatic indices.

**296 Overview of Biological Metrics Evaluated in the Near Shore Environment** R. Ryti, Neptune and Company, Inc.; B. Tiller, Environmental Assessment Services; J. Markwiese, Neptune and Company, Inc., Environmental Assessment Dept; D. Jacques, Washington Closure Hanford. The baseline ecological risk assessment for the River Corridor portion of the Hanford Site in Washington State employed field-, laboratory-, and literature-based lines of evidence to evaluate the potential for adverse effects related to historical releases from plutonium production operations on biota in the near-shore aquatic environment. This paper presents the study design and results for one of the field measures – rock baskets that served as artificial substrates to sample the aquatic community. Clean cobbles were deployed in shallow water (~ 2 meters) at 41 locations along more than 100 km of the Hanford Reach of the Columbia River. This included reference sites upstream of the Hanford Site and study sites along the Hanford Site shoreline. Benthic macroinvertebrates that had colonized the rock baskets were collected after approximately 6 months of deployment and sorted to family, genus, or species level. Because mollusks were of special concern, they were identified to species. Biological information was summarized into 56 total attributes. In addition to characterizing the biota obtained from these rock baskets, habitat and contaminant information was also obtained for the deployment sites. Univariate and multivariate statistical tools were used to determine if there were any overall trends in diversity and abundance and if these trends could be related to habitat, contamination, or some combination of these factors.

**297 Relationships Between Heavy Metals and PCBs in Sediment and Fish Tissue from the Hanford Reach in the Columbia River** L.M. McIntosh, Woodard & Curran, Risk Assessment Services; S. McCarthy, Woodard & Curran; L. Campe, Woodard & Curran, Risk Assessment; C. Trapp,

Woodard & Curran, Risk Assessment Services; L. Hulstrom, Washington Closure Hanford. An approximately 150 mile stretch of the Columbia River in Washington State, upstream, adjacent to and downstream of the Hanford Site (a largely decommissioned nuclear production facility operated by the United States Dept of Energy), has been the subject of environmental investigations for decades. Recently, as part of a remedial investigation of the Columbia River, numerous environmental samples consisting of surface water, sediment, island soils, and different tissue types from several different species of fish, were collected along this river reach. The objective of the study reported here is to ascertain patterns in and relationships among environmental media—specifically, sediment and fish tissue—for a variety of chemical constituents. In particular, in-depth multivariate statistical evaluation was conducted to determine the relationships of select heavy metals (e.g., Hg, Cd, Cu, Mn, Sr and Zn) and polychlorinated biphenyls within sediment as well as relationships of these constituents between sediment and different tissue types of six different fish species. The implications of this approach for using sediment data as an indicator of the uptake of these constituents into fish tissue is also discussed.

**298 An Investigation into the Extent and Biological Impacts of Endocrine Disrupting Chemicals (EDCs) in a Highly Effluent-Dominated River in New England** D. McDonald, K. Kipp, B. Hoskins, T. Faber, USEPA, New England Regional Laboratory; A.L. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; M.A. Mills, USEPA, National Risk Management Research Laboratory; L. Zintek, USEPA, Region 5; J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; H. Haring, The McConnell Group, c/o USEPA ORD; S.F. Nakayama, National Institute for Environmental Studies, Center for Environmental Health Sciences. The Assabet River in central Massachusetts is a heavily effluent-dominated river and, during low-flow conditions, is composed almost entirely of wastewater effluent (i.e., up to 95%). The USEPA Regional New England Laboratory and the USEPA Office of Research and Development Cincinnati Laboratory have been conducting a two-year study to investigate endocrine disrupting chemicals (EDCs) in the effluents and their potential impacts on fish in the river. During the first year of the study, effluents from four wastewater treatment plants were analyzed for a suite of EDCs, steroid compounds, perfluorinated chemicals (PFCs) and other pharmaceuticals and personal care products (PPCPs). Forage fish were collected from the river and analyzed for indicators of EDC exposure, including histopathology and vitellogenin (i.e., egg yolk protein) induction in male fish. Larval whole effluent toxicity exposure tests were conducted using effluents from the plants. Of the 54 pharmaceutical analytes, 29 were found across all four effluents, with six of those having concentrations near 1 ug/L. For the eight steroids measured, one effluent had a concentration of ethinylestradiol at concentrations known to cause effects on fathead minnow fecundity (@2 ng/L). Two plants had PFCs (PFOS/PFOA) at levels near the Minnesota Pollution Control Agency Aquatic Life Criteria. Nonyl Phenols were found at concentrations of at least 5 ug/L in all effluents. Additional results of the first year of research will be presented.

**299 Endocrine Disrupting Compounds in Wyoming Surface Waters: Assessment of Presence, Suspected Sources and Possible Consequences for Wyoming Fisheries** E.O. Johnson, Univ of Wyoming, Dept of Zoology and Physiology; D.T. Pham, Univ of Wyoming, Dept of Zoology and Physiology (3166); J.M. Morris, Stratus Consulting; H.L. Bergman, Univ of Wyoming, Dept of Zoology & Physiology. We initiated this project because of widespread reports from Europe and North America on intersex condition and other reproductive abnormalities in fish related to presence of measured or suspected endocrine disrupting compounds (EDCs) in surface waters (e.g., Joebling et al. (1998) in England on wild roach, Vajda et al. (2008) in Colorado on white sucker, and Hinck et al. (2009) on bass in many US surface waters). These studies often point to discharges from wastewater treatment plants (WWTPs) as the principal sources of natural and synthetic steroids and other chemicals that can affect fish reproduction and fish populations. But WWTP discharges are not the only sources of EDCs. A recent report on trout from remote, high-elevation lakes in the western US point to long-range atmospheric transport and deposition of industrial chemicals such as PCBs as the putative cause of intersex male trout (Schwindt et al. 2009). Possibly a potential issue for Wyoming and other rural agricultural areas are recent studies of livestock manure, including those

associated with rangeland grazing, as a source of steroid hormones apparently causing elevated incidence of intersex male fish (Kolodziej and Sedlak 2007). There appears to be little information about the presence of EDCs from Wyoming surface waters or the possible presence of intersex male fish. But our recent measurement of 11.7 ng/L (parts per trillion) 17-beta-estradiol in the Laramie River above the Laramie WWTP discharge is a particularly concern, given the predicted-no-effect-concentration (PNEC) of 1 ng/L 17-beta-estradiol for protection of aquatic life, as established by the British Environment Agency (Young et al. 2004). Because of this concern, we collected white suckers from the Laramie River approximately 10 miles upstream from Laramie and above any WWTP discharges in this watershed and examined gonadal tissue using H&E stain and light microscopy and observed both oocytes and spermatogenic tissues in the same sections of male fish gonads indicating an intersex condition. We have now expanded this study and will report results on gonadal tissue histology and plasma vitellogenin levels in fish and chemical analyses for EDCs in water samples from four sites along the Laramie River, including (1) a pristine up-river site, (2) a site downstream from potential agricultural runoff, (3) a site downstream from potential septic system releases, and (4) a site downstream from the Laramie WWTP discharge.

**300 Biological and Photochemical Fate of Pharmaceuticals and Their Metabolites in WWTPs, Wetlands, UV and UV/H2O2 Process** E. Lee, Gwangju Institute of Science and Technology, Dept of Environmental Science, GIST, Dept of Environmental Science and Engineering; J. Cho, Gwangju Institute of Science and Technology, Environmental Engineering. As pharmaceuticals have been an emerging concern for several decades, pharmaceutical metabolites are also getting attention due to their potential of ecotoxicological effect. Even though pharmaceutical metabolites have been metabolized in body, some metabolites (10, 11-epoxycarbamazepine, paraxanthine) are still pharmaceutically active and more stable than parent compounds. However, the fate of pharmaceutical metabolites has been rarely known compared to the parent compounds. The purpose of this study is to provide scientific information of behavior of both pharmaceuticals and their metabolites in various water treatment processes. In this study, overall fate of pharmaceuticals and their metabolites was investigated in both biological (WWTPs, and wetlands) and photochemical (UV and UV/H2O2) process. Firstly, transformation of pharmaceuticals into their metabolites was investigated in both WWTPs and constructed wetlands process to understand the environmental fate of pharmaceuticals and their metabolites. Various micropollutant compounds, including 12 pharmaceuticals and 11 metabolites were selected for this study. Whole analytes were measured using Water 2695 Separations Module coupled with a Micromass Quattro Micro triple-quadrupole tandem mass spectrometer in electrospray ionization mode (ESI). Research revealed that most pharmaceutical compounds were substantially eliminated by WWTP process except for atenolol (removal efficiency at 50%) and iopromide (60%). And most of the metabolites (4-OH-diclofenac, hydroxyl-ibuprofen, O-desmethyl-naproxen and CBZ-10OH) were more stable than the parent compounds in both WWTP and wetlands treatment. Well-known pharmaceutically active metabolites (paraxanthine, CBZ-EP and N-acetyl-SMZ) were also detected in treated water. In addition, photochemical fate of pharmaceuticals and metabolites was studied using UV and UV/H2O2 process. Among various pharmaceuticals, diclofenac and sulfamethoxazole showed the highest photodegradation rate. And their pharmaceutical metabolites showed different photodegradation rate compared to parent compounds. 4-hydroxy-diclofenac was more susceptible to the photodegradation than the parent compound, diclofenac. However, N-acetyl-sulfamethoxazole had conservative behaviors during photolysis process in contrast to its parent compound, sulfamethoxazole. This study is expected to be helpful to understand the complex behaviors of pharmaceuticals and their metabolites in aquatic environment.

**301 Endocrine Disrupting Chemicals in a US National Effluent Sampling** M.A. Mills, USEPA, National Risk Management Research Laboratory; S.F. Nakayama, National Institute for Environmental Studies, Center for Environmental Health Sciences; K. Tadele, US Environmental Protection Agency, Student Contractor to National Risk Management Research Laboratory, Student Service Contractor; A.L. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research

Branch; M. Kostich, USEPA Office of Research and Development, EERD. Wastewater treatment plant (WWTP) discharges (both aquatic and solids) are known to contain endocrine disrupting chemicals (EDCs). The long term nature of the discharges and the linkages to in stream effects has been a focus of research in recent years. There have been limited studies to evaluate the scale of the issue and the relationships to the type of treatment received within the WWTP. In, 2010-2011, USEPA ORD conducted sampling and analyses for selected EDCs, pharmaceuticals, and fluorinated chemicals at 50 WWTPs through the United States. The largest municipal wastewater plants in the United States were identified from the 2004 Clean Watershed Needs Survey. These plants produce about 16% of all the municipal wastewater in the US. A one-time grab or 24-h composite sample (total of 9 liters) of finished aqueous effluent from each plant were collected by EPA Regional or WWTP personnel. For each effluent sample, the concentration of steroid hormones, alkylphenolic compounds, and Bisphenol A along perfluorinated compounds and pharmaceuticals, and the estrogenic potential in a fish assay. The EDCs measured were found to have a great range of concentrations and were commonly found in many effluents tested. The result from the study will be discussed in the presentation.

**302 Estrogenic Activity – A Frame of Reference for Some Foods, Agricultural Wastewaters, and Animal Feedstuffs** N.W. Shappell, USDA-ARS-RRVARC, Biosciences Research Lab, USDA-ARS. In the last decade, several in vitro estrogenic activity assays have been used to assess municipal and agricultural wastewaters and surface waters. Some work has been published to provide context by measuring activity from waters collected in pristine or “control” sites, but the relative estrogenic activity of “healthful” foods such as milk, or rice prepared in microwavable bags had yet to be determined. Using the E-Screen – a proliferation assay using untransfected human mammary epithelial cells, estradiol equivalents (E2Eq) were determined for extracts of milk, rice, defatted soybean meal (animal feed), water used to clean chicken eggs from a commercial processing facility (egg wash wastewater) and a 1.25 mg pill of Premarin (used for human hormone replacement therapy). On a serving basis, E2Eq were: skim milk 31 -317 pg (8 oz, 5 different weeks of supermarket collection), vitamin D 4 pg (as fortified in 8 oz of milk), rice 0 - 7303 pg (48 g dry rice, 6 extracts from 4 lots), folic acid 220 pg (as fortified in 48 g rice); with no activity detected from vitamin A, niacin or thiamin (other fortifications in milk or rice). The microwavable bags used to prepare the rice contained 2 - 18 pg of E2Eq per serving (n = 6); no estrogenic activity was found in 3 of 6 rice samples prepared in bags, and in 2 of 6 rice samples prepared without bags. The variability in E2Eq of both milk and rice is to be expected. Milk is a composite product coming from animals at various stages of lactation with variable estrogen secretion. The estrogenic activity in rice is most likely the result of trace mycotoxin contamination, such as zearalenone, and therefore highly variable. To put these values in context, Premarin contained ~300,000 pg/pill of E2Eq and defatted soybean meal used as an animal feed contained ~ 120,000 pg/g dry matter. The highest untreated egg wash wastewater contained 10 ng/L of E2Eq, approximating the proposed lowest observable effect concentration for E2 in fish. Analysis of egg wash wastewater from the holding lagoon indicated a 66% reduction in E2Eq. These data provide a broader context in which to frame past, present and future measures of estrogenic activity.

**303 Fate of Triclosan and Methyltriclosan in Soil from Biosolids Application** N. Lozano, Univ of Maryland, Faculty Research Assistant; A. Torrents, Univ of Maryland, Civil and Environmental Eng.; C. Rice, US Dept of Agriculture, Environmental Management and Byproduct Utilization Laboratory; M. Ramirez, District of Columbia Water and Sewer Authority. Biosolids contain synthetic chemicals that have the potential to alter soil microbial communities and disrupt endocrine functions if they move offsite. In this study, the persistence of triclosan (TCS), an antibacterial compound normally found in biosolids and in soils after biosolids applications was evaluated. The fate of its biodegradation product, Methyltriclosan (MeTCS), was also examined. TCS and MeTCS concentrations were analyzed in biosolids samples collected every two months from a large waste water treatment plant (WWTP) over 4 years. An experimental agricultural plot was treated with a single biosolid application from this plant (average TCS and MeTCS concentrations of  $15.6 \pm 0.6$  and  $0.12 \pm 0.01$   $\mu\text{g g}^{-1}$  dry wt.) and surface soil samples were collected several times afterward, over a three years period. In the experimental plot, for the first 8 months, concentration of TCS in the soil gradually increased with maximum levels of  $63.7 \pm 14.1$  ng g<sup>-1</sup> dry wt., far below the predicted maximum concentration

of 307.5 ng g<sup>-1</sup> dry wt. After one year, TCS disappearance corresponded with MeTCS appearance, suggesting in situ biodegradation. The results suggest that soil incorporation and degradation processes are taking place simultaneously and thus soil concentrations are always far below the predicted maximum concentration. In this study, TCS background levels are achieved within two years. TCS half-life was determined as 104 d and MeTCS half life was estimated to be more than 4 times this value.

**304 Pharmaceuticals, Perfluorinated Compounds and Other Organic Wastewater Compounds in Private Drinking Water Wells on Cape Cod, Massachusetts** L.A. Schaider, Harvard School of Public Health, Dept of Environmental Health, Silent Spring Institute, Dept of Environmental Health; R.A. Rudel, J.M. Ackerman, S.C. Dunagan, J.G. Brody, Silent Spring Institute. The shallow aquifers of Cape Cod, Massachusetts, USA, are vulnerable to contamination from septic systems, the area's predominant form of wastewater treatment, because of the porous nature of the sandy soils and close proximity between septic leach fields and groundwater levels. Such contamination is of concern because groundwater is the sole source of drinking water on Cape Cod. Private wells, which serve 20% of Cape Cod residents, may be more vulnerable to wastewater contamination than public wells because they are located in residential areas without large buffer zones to separate them from septic systems or other sources of groundwater pollution. We tested for 121 organic wastewater compounds (OWCs) in 20 private drinking water wells on Cape Cod. Wells were selected through a two-step screening process. We used GIS land use data to analyze the extent of residential development in a zone near each of 130 wells that were volunteered by local homeowners. Of these, 50 wells were selected for a second round of screening, with the goal of including wells with a range of nearby residential development and geographic locations. Water samples from these 50 wells were tested for two inorganic markers of wastewater, nitrate and boron, and these results were used to select a final list of 20 wells that encompassed a range of concentrations, with an emphasis on wells with high nitrate and boron. We compared the results of OWC testing in these 20 wells to those found in our recent study of Cape Cod public wells and other US source waters. We also evaluated the ability of land use information, well depth and concentrations of nitrate, boron, and acesulfame (an artificial sweetener) to predict overall OWC occurrence. Findings were reported to participating well owners, including supplemental information for interpreting results, and to the general public. Our results provide some of the first results documenting septic system impacts on drinking water supplies and have implications for wastewater management planning and protection of similarly vulnerable water supplies.

**305 Seasonal Variation of Surface Water Veterinary Pharmaceutical Levels in an Agricultural Watershed** D. Snow, Univ of Nebraska-Lincoln, Water Sciences Laboratory, School of Natural Resources, Univ of Nebraska, Associate Professor; D. Rus, US Geological Survey; S. Bartelt-Hunt, Univ of Nebraska, Civil Engineering. Traces of veterinary pharmaceutical compounds can be introduced directly to surface water through illicit or unintentional discharge of livestock wastewater, and indirectly through storm run-off from field receiving wastewater or biosolids application. In areas with a high density of animal feeding operations it is difficult to distinguish between sources and relative importance of these routes. This study investigated the seasonal occurrence of 17 veterinary antibiotics and growth promoters in river and tributaries draining approximately 300,000 acres of cropland with roughly 140 large livestock operations. Polar organic contaminant integrative samplers were used to measure variation of pharmaceutical levels in the watershed at eight locations during spring, summer, and fall in 2008 and 2009. Time-weighted estimated average concentrations of pharmaceuticals were estimated using uptake rates determined in a previous study. Sites with passive samplers were also equipped with continuous monitoring equipment for water levels, conductivity, temperature, and dissolved oxygen. Twelve of the seventeen pharmaceutical compounds were detected in the passive samplers during the monitoring periods. Compounds regularly detected in POCIS extracts include lincomycin, monensin, sulfamethazine, tiamulin, and oxytetracycline. Compounds detected infrequently and at concentrations less than 1 ng/L include ractopamine, sulfachloropyridazine, sulfamethazole, sulfamethoxazole, sulfathiazole, and tylosin. Previous evidence had suggested a spill or direct discharge to surface water, though there was little evidence of a point source release of pharmaceuticals during this study. Run-off from fields fertilized with animal manure is thought to be the major contributor of pharmaceuticals to surface water.



**306 Great Lakes, Great Laboratory: Achievements in Environmental Chemistry** D. Swackhamer, Univ of Minnesota, Environmental Health Sciences. Environmental chemistry was largely motivated by the environmental disasters of the 1950s – 1960s, including air pollution problems in the Ohio Valley, the burning of the Cuyahoga River, and the “death” of Lake Erie. The first account of DDT and PCBs in North America was from fish from the Great Lakes. Many of the visible environmental problems were concentrated in the Great Lakes basin, and much of the research that has helped solve the problems and led to the enactment of many of our federal environmental laws has been done in the Great Lakes. The successes include large scale water quality modeling of phosphorus; analytical advances in POPs measurements; POPs fate and transport, especially the discovery of long-range transport; greater understanding of bioaccumulation; development of large-scale, long term monitoring programs; and more. Each of the problems will be presented along with a case study of how Great Lakes environmental chemistry research has made significant contributions to our understanding of the problems.

**307 Women in Environmental Chemistry: Past Accomplishments, Present Challenges** D. Delach, Clemson Univ, Environmental Toxicology; C.M. Lee, Clemson Univ, Environmental. Engineering and Earth Sciences Dept. Women have been among the pioneers of Environmental Chemistry since its inception. Many women were able to overcome major challenges to make significant contributions that continue to be important to this day. The contributions of scientists such as Ellen Swallow Richard, Alice Hamilton, Rachel Carson, Kathleen Taylor, Rosalyn Yallow, and Susan Solomon will be discussed. Each of the scientists featured contributed to the theory and technology that we use today. These women share some similar characteristics such as passion for science and compassion for humanity that enabled them to overcome challenges and contribute. We will discuss the characteristics and accomplishments of women from the earliest days of environmental chemistry to the present day. We seek to answer how to inspire future chemists to continue the progress in understanding the behavior of contaminants in environmental systems by considering the lives of these women.

**308 Environmental Chemistry: The View From the Cupboard Under the Stairs** A. Jones, Clemson Univ, Institute of Environmental Toxicology, Clemson Univ, Institute of Environmental Toxicology; E. Carraway, Clemson Univ, Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology. What is environmental chemistry? Why is it worth poking my head out from behind the cupboard door? From important chemists of yesteryear, to movers and shakers of today and the budding chemists of tomorrow, this presentation takes a look at how we got here, and where we are going in environmental chemistry from a student's point of view. Stuart Baird and Scott Cann described environmental chemistry as being concerned with the chemical aspects of problems that humankind has created in the natural environment. While that may be a succinct, though pessimistic description, this presentation will explore the origins of environmental chemistry as a reactionary scientific discipline. Our initial lack of understanding of environmental processes has been replaced with advancements in knowledge of the natural world around us. In a short time we have come from ‘the environment will take care of it,’ to ‘dilution is the solution to pollution,’ to the seemingly never-ending search for the limit of our detection capabilities. But where do we go from here? Fortunately, our relentless pursuit of a better understanding of the environment has led to potential remedies to many of our environmental problems. However, as is often the case, issues with economics and applicability at large scales still exist. We still have much work to complete to further our understanding of current and developing environmental problems, to make implementation of solutions less costly, and to better communicate our science to the policy makers and general public.

**309 Equilibrium Partitioning in a Multimedia World: Past, Present and Future** D. Mackay, Trent Univ. Looking back, the author devoted three decades to exploring the feasibility of using the equilibrium criterion of fugacity to complement and help interpret various multimedia concentrations that are measured and used by environmental chemists. Insights that can be obtained by viewing the world of multimedia contamination through the lens of fugacity are outlined, notably fascinating excursions into bioconcentration, air-water exchange, global transport, and the ability to compile very simple and intuitive mass balance models. Coming from a petrochemical

engineering background the author grew to love the fugacity concept but others are understandably less enthusiastic because it is concentrations that are measured and it is doses and body burdens that induce toxic effects: not fugacity. The author was slow to appreciate that the closely related equilibrium criterion of activity has toxicological significance, especially for narcotics, namely Ferguson's “activity hypothesis”. Fugacity can thus describe not only multimedia environmental fate, it can also contribute to assessing effects and risk of effects. Looking forward, there is an opportunity for progress in four areas: improving the determination of equilibrium parameters for a diversity of chemicals experimentally and by computation from molecular structure: testing and improving multimedia models of fate, transport and exposure: testing the “activity hypothesis”: and refining passive sampling methods of determining fugacity and activity directly in the environment and especially within organisms.

**310 The History of Environmental Chemistry – The Mackay Era and Beyond** M.L. Diamond, Univ of Toronto, Dept of Geography and program in planning, Univ of Toronto, Dept of Geography. SETAC was founded 1979, the year Don Mackay started the fugacity legacy with “Finding Fugacity Feasible” (ES&T). The context for the burgeoning field of environmental chemistry was dire reports of global environmental catastrophe from Barry Commoner, and Meadows et al. (“Limits to Growth” 1972) and others. The degraded state of the lower Great Lakes, catalyzed interdisciplinary, problem-solving research amongst chemists, chemical engineers and biologists under the aegis of the International Joint Commission. In this atmosphere Don Mackay, with SETAC colleagues Yalkowsky, DiToro, Eisenreich et al., marshaled the fundamental principles of chemical engineering and chemistry, together with knowledge from other fields such as pharmaceutical sciences and toxicology, to give birth to a new integrated thinking about environmental pollution. The prime innovations by Mackay and SETAC colleagues, which I suggest formed a new paradigm, were explaining that the fate and effects of organic chemicals were predictable, based on knowledge of fundamental physical-chemical properties of the chemical together with an understanding of the environment. I suggest that this was a paradigm shift because this explanation moved the field beyond the phenomenological and towards a consistent, mechanistically-based and unifying theory. Mackay also advocated taking a multi-media approach since, in this new paradigm, organic chemicals were transported and caused effects far from their point of emission – the message introduced by Rachel Carson (“Silent Spring” 1962). It was also a time of the burgeoning of North American universities, with some faculty members hired “sight unseen” and who had time to think and explore science, without the “publish or perish” dictum. In the intervening time, many of us have contributed to fleshing out the “fugacity” paradigm as we do our “normal science” (T Kuhn “The Structure of Scientific Revolutions”). As with any paradigm, inconsistencies mount which, due to our scientific culture, we tend to sweep under the carpet or use to embellish rather than change the paradigm. As dire predictions of global environmental catastrophe mount from the next generation of prognosticators, let's hope that we can give birth to the next paradigm that will meet the challenges that we face!

**311 The Early Days of Environmental Chemistry** R.A. Hites, Indiana Univ, School of Public & Environmental Affairs. Environmental chemistry as a discipline developed from two directions: air pollution and water pollution. As sanitation in urban centers developed in the 19th century, a type of civil engineer evolved; their goal was to understand the processes of treating sewage and drinking water (and keeping the two apart). These folks became known as sanitary engineers and had skills in both hydrology and in microbiology. At the same time, people realized that air pollution was a significant problem, particularly in highly industrialized cities, and dealing with these problems lead to some fundamental work on gas-phase chemical kinetics. In parallel and crystallized by Rachel Carson's 1962 book *Silent Spring*, interest also developed in what were at the time called “pesticide residues.” The work from these three rather diverse groups was being published in different journals. The sanitation engineers were typically publishing in water treatment journals and presenting papers at civil engineering meetings. The atmospheric scientists were publishing and presenting at earth sciences meetings. The pesticide chemists were publishing in agricultural journals and presenting at food science meetings. In many ways, this was reasonable given that the intellectual and measurement tools used by these three populations were quite different. In the 1960s, the American Chemical Society had a division called Air, Water, and Waste Chemistry – an accurate but unappealing title. By

the time of the first Earth Day in 1970, this division had changed its name to Environmental Chemistry with the goal of being inclusive. About the same time the American Chemical Society's journal Environmental Science and Technology was founded. These two initiatives solidified environmental chemistry as a real discipline, at least among chemists. The Society for Environmental Toxicology and Chemistry (SETAC) was founded in 1979, and again the goal was to be inclusive and to reach out to applied aquatic toxicologists (on the one hand) and environmental chemists (on the other). Initially this inclusiveness was less than fully realized; for example, the first SETAC meeting I attended in 1984 had one half-day session devoted to all aspects of environmental chemistry. Eventually, through the hard work of SETAC's Board of Directors, the annual meeting began to attract more chemists, and it is now one of the annual highlights of the scientific conference calendar.

**312 Proteomic Analysis of Colorful Oysters from Metal Contaminated Estuary** F. Liu, The Hong Kong Univ of Science & Technology; W. Wang, Hong Kong Univ of Science and Technology. Proteomic analysis of ecologically and economically important organisms is a promising novel approach for environmental toxicology. Few proteomic studies have been applied to natural contaminated ecosystems. We collected three populations of oysters along a metal-contaminated estuary together with one population as the control from a nearby pristine site. Strikingly, tissue color changed markedly from white (control) to yellow, green and blue, and the increasingly darken colors were in good agreement with a steady increase in measured metal concentrations (Cd, Cu and Zn) in the gills. Proteomic analysis of the gills as one of major metal targets suggested that metal overload led to systemic dysfunctions in metabolisms of reactive oxygen species, amino acid and protein, lipid peroxidation, cytoskeleton, and energy production. These molecular alterations were responsible for the visible population abnormality (e.g., tissue coloration). Meanwhile, we found a unique protein expression signature composed of 11 altered proteins, and the signature could successfully distinguish the population from clean and contaminated areas and achieve perfect classification of the contaminated animals with different levels of metals mixture exposure. More importantly, the expression abundances of 8 proteins, i.e., 26S protease regulatory subunit 6A, actin, CRE-PGK-1, elongation factor 2, enolase, gelsolin, mitochondrial H<sup>+</sup> ATPase a subunit and an unnamed protein, were significantly related to gill Cu, Zn or Cd concentration. These proteins could be very useful as novel biomarkers in environmental pollution assessment.

**313 Metabolic Profiling of Calanus Finmarchicus to Study the Impact of Waterborne Diethanolamine Exposure** K.E. Degnes, SINTEF, Materials and Chemistry; T.R. Stoereth, SINTEF, Fisheries and Aquaculture; D. Altin, BioTriX; H. Sletta, K. Zahlsen, S.F. Borgos, B. Hansen, SINTEF, Materials and Chemistry. The metabolic impact of exposure to different concentrations of the alkanoamine diethanolamine (DEA) on the microcrustacean *Calanus finmarchicus* was evaluated. *C. finmarchicus* is one of the most important organisms in the North Atlantic Ocean due to its significant position in the food web. This crustacean has therefore been used as a relevant model for a numerous eco-toxicity tests performed at SINTEF/NTNU. Thus a large database of eco-toxicological data exists for this species. DEA has low acute toxicity (96 hrs-LC50=380mg/L), but due to the widely use of this solvent in a considerable number of industrial applications, it is relevant to elucidate potential sub-lethal aquatic eco-toxicological effects of this amine. DEA is therefore a suited model chemical for metabolic fingerprinting and metabolic profiling of *C. finmarchicus*. We have previously published that DEA has an effect on the *C. finmarchicus* metabolome using 1H nuclear magnetic resonance spectroscopy (NMR). In the present study we wanted to further investigate if liquid chromatography mass spectroscopy (LC-MS) based metabolomics could uncover sub-lethal stress responses in *C. finmarchicus* that would not have been observed with classical toxicological tests. LC-TOF and Mass Profiler Professional software were used as diagnostic tools in order to evaluate molecular stress responses in the test species. Metabolic fingerprinting with PCA analysis revealed changes in metabolite profiles at concentrations far below LC50, and these results were confirmed by NMR. Putative metabolites were identified and metabolites related to biosynthetic pathways that are likely to be significant for important physiological responses were subject for further investigations. We have in particular investigated metabolites related to the glycerophospholipid metabolism. Preliminary data indicate that the metabolic profiles of several classes of glycerophospholipids are altered in response to DEA

exposure, but work remains in order to investigate if these changes have biological relevance for *C. finmarchicus*.

**314 Assessment of Cadmium Toxicity in the Liver and Gonads of Adult Largemouth Bass (*Micropterus salmoides*) Using Microarrays** A.C. Mehinto, K.J. Kroll, D.S. Barber, N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. Cadmium is one of the most common pollutants found in Superfund sites. This heavy metal can accumulate in the aquatic environment and interfere with fish reproduction. Research in mammals also demonstrated that cadmium can induce hepatotoxicity. This study examined the acute physiological changes induced by a low dose of cadmium (20 µg/kg) in the liver and gonads of adult male largemouth bass. A 15K largemouth bass microarray was used to examine the genes differentially regulated in both tissues. Microarray analyses revealed that the genomic responses identified were tissue-specific with more genes being affected in the liver than in the gonads of the male fish. In the liver many genes related to DNA repair and transcription were down-regulated. Oxidative stress response and a disruption of carbohydrate metabolism were also observed in the liver. Fewer genes were differentially expressed in the gonads. Gene Ontology analysis showed that lipid metabolism and steroid biosynthesis were significantly altered. In this tissue Transcription-related processes were disturbed in the gonads but the genes identified were dissimilar to those found in the liver. Although the main biological processes disturbed by cadmium exposure can equally be affected by other environmental toxicants, some of the genes identified in this study have been consistently affected by cadmium and they could be considered as potential biomarkers of cadmium exposure.

**315 The Cytochrome P450 Superfamily in the Rotifer, *Brachionus orientalis*: Annotation and Their Transcript Expression Analysis** R. Kim, Hanyang Univ, Dept of Chemistry; J. Lee, Hanyang Univ, Dept of Chemistry, Hanyang Univ Graduate School, Dept of Chemistry. Cytochrome P450 (CYP) genes, a large and most versatile gene family, translates essential enzymes for the detoxification of xenobiotic chemicals and the metabolism of drugs. To date, CYPs and those of specific activities have been characterized in numerous invertebrates. However, CYPs showed a considerable sequence divergence in allied species and little information is available about their innate functions. For these reasons, a study on CYPs may provide us with an opportunity to elucidate specific functions and regulation mechanisms of particular CYP genes. Since the rotifer *Brachionus* sp. represents an underived metazoan species we expect it to provide evolutionarily primitive forms of the whole CYP complement genes. This also makes it a suitable model species for marine toxicity testing. To annotate *Brachionus* sp. CYPs, we constructed phylogeny trees using Mr.Bayes v3.1.2 program. CYPs from *Brachionus* sp. were divided into five distinct clans (clan 2, 3, 4, 46) and mitochondrial groups (8 families, 8 subfamilies, and 28 isoforms). Clan 2, 3, and the mitochondrial groups showed close similarity with those of other invertebrates, whereas Clan 4 and 46 more similar to vertebrates. To analyze the effect of B[α]P as a strong inducer of cytotoxic stress, we exposed 10, 100, and 1000 µg/L B[α]P for 24 h and measured transcript profiles by quantitative real-time RT-PCR. Upon B[α]P exposure, CYP isoforms including Clan 4, 46, and mitochondrial groups showed no significant mRNA expression. However, other isoforms of Clan 2 and 3, showed a significant up-regulation in a concentration-dependent manner. These results indicate that these two isoforms may have an important role on the metabolism of xenobiotics like B[α]P in *Brachionus* sp. For further investigation of CYP genes we measured the mRNA expression of CYP isoforms (Clan 2 and 4) after exposure to a WAF (Water Accommodated Fraction) that was mainly composed of PAH from heavy oil spillage in the Yellow Sea of Korea. We also obtained the relationship of those CYP genes in the AhR-CYP transduction pathway. The present study may provide comprehensively annotation of the rotifer cytochrome superfamily and indicates their putative role in the mechanism of detoxification of environmental toxicants.

**316 Use of Genome and Proteome Analysis to Reveal Toxicity Pathways in Rainbow Trout Exposed to the Brominated Flame Retardant TBBPA-DBPE** A. Muttray, D. Simmons, T. Neheli, S. Clarence, J. Miller, J. Sherry, Environment Canada, Water Science & Technology Directorate. Fluorescent RNA Arbitrarily Primed (FRAP)-PCR is an open-ended exploratory tool that identifies gene expression differences without prior knowledge of the affected genes. We employed FRAP-PCR to select genes that clearly responded to Tetrabromobisphenol A bis(2,3-dibromopropyl

ether) (TBBPA-DBPE) in rainbow trout (*Oncorhynchus mykiss*) liver. The flame retardant TBBPA-DBPE was introduced as a replacement for other high-volume brominated flame retardants that showed clear evidence of carcinogenicity. Although little toxicity data is available for TBBPA-DBPE, related chemicals have been found to be endocrine-disrupting, geno-, hepato-, immuno- and neuro-toxic in some tests. For risk assessment purposes, it is important to learn if genetic pathways linked to adverse outcomes are affected by this flame retardant. About 100 FRAP-PCR amplification products were identified as differentially expressed, extracted from the differential display gels, and sequenced. Approximately half of the FRAP-PCR products were positively identified using the BLAST algorithm. The sequenced and identified products were entered into Ingenuity Pathway Analysis software (IPA) to categorize them into biochemical pathways. Key pathways and network nodes affected by TBBPA-DBPE were confirmed by qPCR. Affected pathways included the acute phase response, cell cycle, protein metabolism, and genes for serum albumins. qPCR confirmation of the remaining network nodes and differentially expressed genes is in progress. Similar real-time PCR analysis of hepatocyte cultures exposed to TBBPA-DBPE allowed us to compare in vitro to in vivo gene expression signatures. To juxtapose genome-level responses with responses at the proteome level we performed a qualitative protein profile of hepatocyte and plasma samples by liquid chromatography quadrupole time-of-flight (LC-Q-TOF) mass spectrometry. Approximately 12% of filtered Q-TOF spectral data were positively identified as peptides from proteins in hepatocyte lysates using the Agilent Technologies SpectrumMill protein database search software. IPA indicated that proteins involved in cellular development, function, maintenance, assembly and organization were found in all treatments and controls. TBBPA-DBPE-treated hepatocytes, but not controls, also expressed proteins significantly linked to protein networks involved in endocrine system disorders, molecular transport, and nucleic acid metabolism.

**317 Benzene and Trichloroethylene Induce Common Mechanisms of Toxicity in Largemouth Bass** R.C. Colli-Dula, A. Alvina Mehinto, Univ of Florida, Center of Environmental and Human Toxicology; A. Cuenca Navarro, IBMB-CSIC, Dept of Molecular Biology; K.J. Kroll, D. Barber, Univ of Florida, Center of Environmental and Human Toxicology; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. Benzene and trichloroethylene (TCE), known environmental pollutants, have been detected in aquatic systems causing adverse effects in fish. In recent years genomic tools, like microarrays, have been useful to quantify gene expression, allowing the exploration of mechanisms of toxicity and the identification of biomarkers of exposure and effect. The object was to use microarrays to investigate changes in gene expression caused by exposure to benzene and TCE in largemouth bass. Forty eight fish were individually weighed and injected IP with a single dose of benzene and TCE (10 mg/kg b/w). After injection, the fish were sacrificed at 48h. Livers and gonads were obtained and analyzed for gene expression changes using microarrays. The hypothesis was that concentrations of 10 mg/kg of benzene and TCE would produce changes in sensitive genes. In the liver 1332 and 1236 genes were significantly affected ( $p$

**318 Connecting Triclosan's Impact on the Swimming Behavior of Larval Fathead Minnow (*Pimephales promelas*) with Altered Ryanodine Receptor Pathways** E.B. Holland Fritsch, Univ of California- Davis, Molecular Biosciences; R.E. Connon, Univ of California – Davis, School of Veterinary Medicine, Aquatic Toxicology Laboratory; I. Werner, Swiss Centre for Applied Ecotoxicology, Eawag/EPFL; I.N. Pessah, Univ of California, Davis, Molecular Biosciences. The antibacterial agent triclosan is commonly found in consumer products including toothpaste, hand soap, and textiles. As an emerging contaminant of concern found in countless environmental samples around the world, including wild caught fish tissue and dolphin plasma, more and more research is beginning to address its impacts in vertebrate species. Notably, recent research in mammalian cells has shown that triclosan alters  $Ca^{2+}$  signaling through the ryanodine receptor (RyR), important for muscle function. Altered RyR functioning, due to triclosan, has not been investigated in teleost species but could have vast effects on the overall health and behavior of an organism. To address these effects we evaluated time-dependent and dose-dependent impacts of triclosan on survival and swimming behavior in correlation with altered RyR related gene expression, protein levels and protein functional states. The nominal 7d median lethal concentration (LC50) was 193 $\mu$ g/L-1. Triclosan had a significant impact on swimming behavior and ability after 1d that was consistent after 4d and

7d of exposure. There was a time-dependent and dose-dependent gene expression response to the antibacterial agent, which significantly altered the expression of the three RyR isoforms (*RyR1*, *RyR2*, and *RyR3*), as well as, several genes that tightly regulate RyR activity; *Dihydropyridine receptor*, *Selenoprotein-N* and *Glutathione-Sulfur-Transferase*. Interestingly, triclosan did not increase RyR activity in whole fish homogenates suggesting that RyR related impacts are not direct actions on the receptor. Our research increases the understanding of triclosan's mode of toxicity across vertebrate species and begins to address RyR dysfunction in fish due to environmental contaminants, which to date has only been investigated in mammals.

**319 A Stress-Responsive Whole-Cell Array for Comprehensive Ecotoxicity Analysis** C. Gao, N. Gou, A. Onnis-Hayden, A. Gu, Northeastern Univ, Civil and Environmental Engineering. Current toxicity evaluation methods like whole effluent toxicity testing (WET) and toxicity identification evaluation (TIE) require sophisticated and laborious procedures, as well as long time for the test. It is desirable to develop fast and reliable assessment methods to detect the presence and effects of toxicants. In this study, we utilized a whole-cell array in the development of methodology in the quantitative measurement of the toxicological effects caused by toxicants, and in the differentiation of toxicants by their toxicological mechanisms through bioinformatic analysis. The array consists of a library of *Escherichia coli* K12 strains carrying gene promoters with transcriptional *gfp* fusions, covering 10 categories of known stress responses. We selected 11 potential toxicants ranging from model chemicals such as Mitomycin C (MMC) and 4-Nonylphenol (4-NNP), to newly emerging nanomaterials such as nano-silver (nAg) and single-walled nanotube (SWNT). The real-time gene expression levels under the exposure of these chemicals were monitored over a period of 2 hours, and transformed into a newly developed Transcriptional Effect Level Index (TELI) to reflect the toxicological impacts. The results shows the TELI values follow a dose-response pattern and fit logistic regression model well ( $R^2$  values near 0.9). Thus we derived endpoints such TELI<sub>50</sub> and TELI<sub>NOTEL</sub> analogous to EC50 and NOTEL, finding close relationship among some of them. It also shows that the contributions from different categories of genes to overall TELI value are not equal, namely the pattern of gene expression distribution is distinct among chemicals. These results indicate a possibility to differentiate chemicals according to their TELI patterns. We applied clustering methods such as Hierarchical Clustering (HCL) and Self-Organizing Maps (SOM) to the samples of different chemicals with changing concentrations, and found that 1) the samples with extreme low or high concentrations of chemicals could be clustered together, without distinguishing the chemical species, implying two ends of toxic effects; 2) the other samples tend to group into subsets containing similar chemicals, 3) the subsets with similar toxicological mechanisms tend to group together. In conclusion, our whole-cell array could be used to measure the toxic effect of toxicants and explore their mechanisms accordingly.

**320 Growth and Bioaccumulation of Copper in Barley (*Hordeum vulgare*) Exposed to Copper Oxide Nanomaterials** H. McShane, McGill Univ, Natural Resource Sciences, McGill Univ, Dept of Natural Resource Sciences; J.K. Whalen, McGill Univ; G.I. Sunahara, NRC-Biotechnology Research Institute, NRC-Biotechnology Research Institute; W.H. Hendershot, McGill Univ, Dept of Natural Resource Sciences. The replacement of micron-sized materials with nano-sized materials in commercial applications can increase product efficiency and reduce manufacturing costs. However, it is necessary to ensure that the subsequent release of these novel materials into the environment, either intentionally or unintentionally, has no negative impact on exposed organisms. The current study will present results of tests in which barley seedlings are exposed to copper oxide materials in agricultural field soil. Commercially-available copper oxide materials with different primary particle sizes, ranging from nano- to micron-size, and variable specific surface area, are used to spike the soils. We report the effects of primary particle size and specific surface area on barley root and shoot length, total biomass and bio-accumulation of copper. We also report the effect of the copper oxide on selected soil chemical properties relevant to model copper transformations in the plant-soil system. These results will be discussed in the context of current guidelines that regulate copper concentrations in agricultural soils.

**321 Nanomaterial Contamination of Agricultural Plants** J.C. White, C. Musante, J. Hawthorne, Connecticut Agricultural Experiment Station, Dept of Analytical Chemistry. Although the use of nanomaterials (NM)



has increased dramatically in the past decade, the potential risks posed to humans and the environment have been investigated only recently. A number of studies evaluating nanomaterial exposure to a range of receptors have been published but there is little information about the effects on agricultural plants. We have previously shown that standard phytotoxicity tests such as germination and root elongation are not sensitive enough or appropriate when evaluating NM toxicity to terrestrial plant species and that plant growth assays are preferred. Different *Cucurbita pepo* subspecies were exposed to 100-1000 mg/L elemental (Ag, Cu, Si, Au) or carbon-based nanomaterials (fullerenes, single and multi-walled nanotubes), as well as corresponding bulk material controls, in Hoagland's solution amended with or without humic acid (100 mg/L). Plant biomass, transpiration, solution content, and particle accumulation were determined. Nanoparticle Ag and Si reduced plant biomass and transpiration by 30-91% compared to equivalent bulk materials. In addition, the accumulation of Ag and Si was significantly greater for nanoparticles than for the corresponding bulk particles. Au had no effect on plant growth, regardless of particle size. Fullerenes had no impact on plant growth in Hoagland's solution but the presence of humic acid in solution resulted in significant reductions in transpiration volume. Our data clearly demonstrates differential toxicity and accumulation of several nanomaterials relative to respective bulk particles. The finding solution chemistry differentially impacts bulk and nanomaterial behavior highlights the importance of evaluating NM fate and effects under environmentally relevant conditions.

**322 Effects of Metal Oxide Nanomaterials on Soil Microbial Populations and Communities: Observations and Explanations** P. Holden, Y. Ge, J. Priester, R. Vukanti, A. Horst, O. Brun, J. Schimel, Univ of California. With their widespread manufacture and use, engineered nanomaterials (ENMs) are increasingly expected to be transported into soils where they may accumulate and affect soil bacterial communities that importantly catalyze critical reactions in nutrient cycling. Questions regard the potential for ENMs to negatively affect soil microbes, and the degree to which effects originate in either indirect changes to soil properties or direct effects on cells. In this study, we exposed California grassland ecosystem surface soils to varying concentrations of nanoTiO<sub>2</sub> and nanoZnO. We assessed the potential for direct effects to cells under controlled, well-mixed, conditions where ENMs are fully bioavailable. We assessed the potential for altered soil water characteristics as a measure for indirect effects of ENM addition related to water retention. We used community profiling of genes encoding 16S rRNA to observe shifts in diversity and composition after 15 and 60 days. Taxa that shifted were identified by pyrosequencing. Through this study, specific taxa, many of which are functionally important, were decreased in abundance with increasing ENM exposure and over time. Water characteristics in TiO<sub>2</sub>-amended soil were examined, as well as ENM effects on cell growth, to inform the relative importance of direct, versus indirect, effects of ENMs on soil bacterial community structure and function. Additional possible indirect effects of ENMs to soil bacterial communities are discussed, as well as their relative importance to altering microbial communities.

**323 Toxicity Testing of ZnO Nanoparticles with Soil Organisms: The Case-studies in NanoFATE, a FP7 EU Project** S. Loureiro, Universidade de Aveiro, Dept of Biology & CESAM; P. Tourinho, A.M. Soares, Univ of Aveiro, Dept of Biology & CESAM; W. Tyne, L. Heggelund, M. Diez-Ortiz, D. Spurgeon, C. Svendsen, NERC-Centre for Ecology and Hydrology; K. Jurkschat, Oxford Univ, Dept of Materials; P.L. Kool, C.A. Van Gestel, Vrije Universiteit, Dept of Animal Ecology. Application of Engineered nanoparticles (ENP) in certain products means they have a route into the environment. In case of metal based ENP, like the ones studied in NanoFATE (EU FP7 project), interaction with natural components, dissolution and agglomeration will be important factors affecting their bioavailability and possible effects. While nano-dependent behaviours of ENP may be desirable for several commercial and industrial purposes, it remains difficult to foresee their potential implications for how the fate and effects of ENP in the environment may differ from those expected from metals in ionic form. Metal oxide-based ENPs, such as ZnO, used in personal care or cleaning products will enter waste water treatment plants (WWTP), and pass to soil systems upon land application of sewage sludge. Hence, knowledge on how to assess ENP toxicity to soil organisms is crucial for developing the environmental hazard assessment of these emerging substances. In this work we are presenting some case-studies of ZnO-NP exposures to different soil organisms with different life traits in natural soil. Modifications needed to dosing

procedures and exposure validation to work confidently with nanoparticles will be covered. These include inclusion of different "control treatments" so ZnO-NP toxicity could be compared with the correspondent non-nano equivalent ZnO and with ZnCl<sub>2</sub>. In initial tests with *Eisenia fetida*, the reproduction EC50 for ZnO-NP was observed around 700mg Zn/kg, while the ionic Zn form (ZnCl<sub>2</sub>) EC50 was found at 500mg/kg. Also for the collembolan *Folsomia candida*, 28-d EC50 values based on Zn concentrations in soil were lower for ZnCl<sub>2</sub> exposures compared to ZnO-NP; but EC50 values based on free Zn in the soil pore water were in the same range for both ZnO-NP and ZnCl<sub>2</sub> (10-16 mg Zn/l), suggesting that toxicity is mainly due to free zinc. For the isopod *Porcellionides pruinosus*, oxidative stress biomarkers responded to ZnO-NP exposures in soil. Although there is a lack of information on the predicted environmental concentrations for ZnO-NP, the case-studies with these three relevant soil organisms provide useful information for future hazard assessment of ZnO-NP. However, other studies undertaken in the NanoFATE project prove that once testing moves beyond strict standard conditions and short time frames (such as pH ranges and ageing) unforeseen and hard to explain patterns may be found in ENP fate and effects within soil systems.

**324 <sup>1</sup>H NMR Metabolomics as a Non-targeted, Molecular Level Technique to Detect Sub-lethal Earthworm Responses to Titanium Dioxide Nanoparticles in Soil** M. Whitfield Aslund, Univ of Toronto, Physical and Environmental Sciences; H. McShane, McGill Univ, Natural Resource Sciences, McGill Univ, Dept of Natural Resource Sciences; M.J. Simpson, A.J. Simpson, Univ of Toronto, Physical and Environmental Sciences; J.K. Whalen, McGill Univ, Natural Resource Sciences; W.H. Hendershot, McGill Univ, Dept of Natural Resource Sciences; G.I. Sunahara, NRC-Biotechnology Research Institute, NRC-Biotechnology Research Institute. Traditional ecotoxicity tests rely on organism-level endpoints (i.e., survival, growth, or reproduction) but may be insufficient to predict long-term population level toxicity (e.g., immune system impairment or accumulation of DNA damage over multiple generations). Therefore, various molecular- and/or cellular-level toxicity tests have been developed to provide sensitive measures of sub-lethal organism responses to contaminants. One such emerging technique is environmental metabolomics, which provides insight into the biochemical response of an organism to an environmental stressor by comparing the concentrations of endogenous metabolites in a tissue or biofluid. Changes in the metabolic profile can reveal information about the organism's response to contaminant exposure at the metabolic level as well as the contaminant's toxic mode of action. In this study, <sup>1</sup>H NMR-based metabolomics was used to examine the response of *Eisenia fetida* earthworms raised from juveniles to sexual maturity (20-23 weeks) in soil spiked with either 20 or 200 mg/kg of a commercially available uncoated TiO<sub>2</sub> nanomaterial (nominal diameter, 5 nm). To distinguish responses specific to particle size, soil treatments spiked with a micron-sized TiO<sub>2</sub> material (nominal diameter, < 45 µm) at the same concentrations (20 and 200 mg/kg) were also included in addition to an unspiked control. Multivariate statistical analysis of the <sup>1</sup>H NMR spectra for *E. fetida* tissue extracts suggested that earthworms exhibited significant changes in their metabolic profile following TiO<sub>2</sub> exposure for both particle sizes and that this response was less variable when the TiO<sub>2</sub> concentration increased from 20 to 200 mg/kg. In general, the observed earthworm metabolic response to both materials was compatible with oxidative stress, which has been proposed as a mechanism of toxicity related to nanoparticle exposure. In contrast, a parallel study observed no differences in earthworm survival, reproduction, or growth following exposure to the same nano-sized TiO<sub>2</sub> spiked soil. This suggests that metabolomics may be more sensitive than traditional ecotoxicity endpoints for detecting earthworm sub-lethal responses to TiO<sub>2</sub> nanoparticles in soil and that further molecular or cellular level assays (e.g., DNA damage, immune function) should be considered to better assess organism responses to TiO<sub>2</sub> nanoparticle exposure.

**325 Ecotoxicity of Silver Nanoparticles on Stress Response Gene Expression in *Chironomus riparius*** P.G. Nair, S. Park, Univ. of Seoul; J. Choi, Univ of Seoul, School of Environmental Engineering. The increased use of silver nanoparticles (AgNPs) in various products makes it likely to result in the release of AgNPs into the aquatic environment, which finally leads to their accumulation in sediment. *Chironomus riparius*, an aquatic midge (Chironomidae, Diptera), is extensively used as a model organism in sediment toxicity. In this study, toxicity of AgNPs was investigated in *C. riparius* using expression of stress response genes, identified from Expressed

Sequence Tags developed by 454 pyrosequencing. The selected genes were Heat Shock Protein 70, Transferrin, Heme oxygenase-1, Cytochrome P4501A1, Quinone Oxidoreductase, Glutathione S-Transferase, Superoxide Dismutase, Catalase, Glutathione Peroxidase, and Start1 genes. The responses of these genes to AgNPs exposure were compared with higher level effects, such as, development and reproduction. Overall results suggest that AgNPs lead significant alteration on genes of various biological functions, and some of them were found to be correlated with alteration on population level effects. This work was supported by the Ministry of Education, Science and Technology of Korea [2010-0027722].

**326 Toxicity of Fullerenes (C<sub>60</sub>) in Sediments** E.J. Petersen, NIST, Biochemical Sciences, National Institute of Standards & Technology; K. Pakarinen, G. Leinonen, Univ of Eastern Finland; M.T. Leppanen, J. Aikonen, J. Kukkonen, Univ of Eastern Finland, Dept of Biology. Effects of fullerene-spike sediment on two benthic organisms, *Lumbriculus variegatus* (Oligochaeta) and *Chironomus riparius* larvae, were investigated. Fullerene agglomerates were prepared using a water stirring method. *L. variegatus* were exposed to 10 and 50 mg fullerenes/kg sediment dry mass for 28 d. These concentrations did not impact worm survival or reproduction compared to the control. Feeding rates were also slightly decreased for both concentrations indicating fullerenes' disruptive effect on feeding. *Chironomus riparius* were exposed after allowing suspended fullerenes to settle down creating a layer of nC<sub>60</sub> on top of the sediment, another environmentally realistic exposure method. Two food concentrations were tested (0.5 % and 0.8 % *Urtica* sp.) in sediment containing fullerene masses of 0.36 to 0.55 mg/cm<sup>2</sup> using a 10-d chronic test. In the 0.5 % food level treatment, there were significant differences in all growth-related endpoints for fullerene exposed organisms compared to controls. Fewer effects were observed for the higher food treatment. Fullerene agglomerates were observed by electron microscopy in the gut but no absorption into the gut epithelial cells was detected in either organism. Electron micrographs of *L. variegatus* also indicated that 16 % of the epidermal cuticle fibers of the worms were destroyed in the 50 mg/kg exposures. For *C. riparius*, gut microvilli were damaged and significantly shorter.

**327 Method Development for Detecting Ag Nanoparticles in a Soil Using AF4 and ICP-MS** A. Whitley, Univ of South Carolina- Aiken, Chemistry and Physics, Univ of Kentucky, Plant and Soil Sciences; J.M. Unrine, Univ of Kentucky, Dept of Plant and Soil Sciences, Univ of Kentucky, Dept of Plant & Soil Sciences; P. Bertsch, Univ of Kentucky, Plant and Soil Sciences. Products containing manufactured nanoparticles continue to increase in use with silver as a main product choice, largely due to its antimicrobial properties. Normal use of silver nanoparticle containing products may lead to release of silver nanomaterials into wastewater streams. Due to the partitioning of particles during wastewater treatment, sewage sludge is proposed to be a main reservoir of Ag NPs. Therefore, land application of sewage sludge biosolids creates increased concentrations of Ag NPs on agricultural soils, leading to increased exposure to soil dwelling organisms. Unfortunately, few techniques are available for the in situ detection and characterization of manufactured nanomaterials in soils. We have developed a method for detecting Ag NPs in a sandy loam soil which includes pore water extraction techniques as well as optimizing parameters for particle size separation and analysis. Asymmetrical flow field flow fractionation (AF4) was used to separate different size particles extracted from the soil pore water, then coupled to UV-vis diode array detection, multi-angle/dynamic light scattering (MALLS/DLS) and inductively coupled plasma mass spectrometry (ICP-MS) in order to characterize Ag containing colloids based on size. Various pore water extraction techniques have been investigated to ensure Ag NP aggregation state is not altered when extracted from the complex soil matrix. Investigations have also been underway to ensure that AF4 parameters including membrane type, external field and carrier composition do not alter the aggregation state of the colloids. Development of methods for detection and characterization of Ag NP partitioning between solid phases and soil pore water is a stepping stone to understanding Ag NP behavior and transformation within soils.

**328 Modernizing Requirements for the Ecological Assessment of Chemicals: The Move to Intelligent Testing Strategies** M. Bonnell, Environment Canada, New Substances Division, Environment Canada, Science and Risk Assessment Directorate. The generation of data for the regulatory assessment of chemicals is undergoing a paradigm shift. Traditional

toxicological methods that use a large number of animals is being replaced with alternative test methods. Mostly, this has been driven by the fact that government and industry are now required to examine large numbers of chemicals in their inventories for hazard properties using a relatively rapid assessment approach. Fewer chemicals undergo the long assessment cycles traditionally associated with legacy chemicals such as PCBs, mercury and dioxins. The focus of regulatory assessors today is on lesser studied practically unknown chemicals with little or no data, let alone toxicological data. One can imagine then the animal usage, cost and time required to test thousands of chemicals in an inventory in order to satisfy traditional data requirements for hazard or risk assessment. Animal rights activists, particularly in Europe, have petitioned the European Commission to severely reduce or even abandon animal testing for the data requirements under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals). Intelligent or integrated testing strategies (ITS) have therefore evolved to help fulfill data needs for modern regulatory assessment with the intent of reducing animal usage. The focus of these strategies has been on toxicity testing, but strategies also exist for other endpoints in risk assessment (e.g., bioaccumulation and biodegradation). This presentation will examine the critical tiers of an ITS and discuss how elements within each tier, such as in silico and in vitro methods interact to help satisfy data requirements for chemical assessment yet also provide a weight of evidence. ITS concepts shall be presented using bioaccumulation assessment as an example.

**329 Revisiting the Relationships Between the Fish Embryo Toxicity Test and the Acute Fish Toxicity Test (OECD 203) – New Data, New Insights** S.E. Belanger, The Procter & Gamble Company, Environmental Stewardship Organization, The Procter & Gamble Company, Central Product Safety Dept; F. Busquet, European Commission JRC/ IHCP/ ECVAM; T. Braunbeck, Univ of Heidelberg, Dept of Zoology; G. Carr, Procter & Gamble; M. Halder, European Commission JRC/IHCP/ECVAM; A. Lillicrap, NIVA; J. Rawlings, Procter & Gamble; R. Strecker, Univ of Heidelberg, Dept of Zoology; S. Walter-Rhode, UBA, German Federal Environmental Agency; A. Gourmelon, P. Amcoff, OECD. The OECD 203 Acute Fish Toxicity Test Guideline (TG 203) is used as a basis for chemical safety assessments worldwide. In 2005, the German Federal Environment Agency submitted a draft TG on "Fish embryo toxicity (FET) test" to the OECD Test Guideline Program and a supportive Background Paper. Subsequently, OECD established the ad hoc Expert Group on the FET Test. The Expert Group concluded that critical components to establish the utility of the Fish Embryo Toxicity Test (FET) as an alternative to the (OECD 203) included better understanding of inter- and intra-laboratory variability of the method, the ease of method transfer, and the relationship between the FET and acute fish toxicity (OECD 203). The FET-acute fish relationship was previously examined by Lammer et al. (2009); however, additional information on the domain of applicability was needed as well as the influences of FET inter-laboratory variability. The OECD established a Validation Management Group for the FET, coordinated by ECVAM, to oversee a comprehensive international validation involving several laboratories. Significant advances in knowledge in the key areas have been gained along with a burgeoning data base of new FET results for additional chemicals. We revisit the FET-acute fish toxicity relationships incorporating results from the first of this two-phase validation program. FET Inter-lab variability measured as coefficient of variation for seven organic compounds that ranged in toxicity between 0.3 mg/L to 12 g/L (4+ orders of magnitude) was demonstrated to be between ~5 to 50%. Reproducibility was fairly independent of toxicity, but driven by chemical properties. In revising the FET-acute Fish relationship we investigate the influence of 48-hr FET data (much of the historical studies) versus 96-hr FET data (more recent). An increase in the chemical coverage from the historic relationship (n= 77 chemicals) to the current update (~ 100 chemicals) reflects a broad change in the applicability domain. The new data results in marginal change to the older FET-fish regressions while addressing concerns about a lack of knowledge in the FET axis. Example of species-specific relationships (e.g., zebrafish only data) versus pooled species relationships will be also be reviewed. "Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries".

**330 Assessment of In Silico Methods to Estimate Aquatic Species Sensitivity** M.G. Barron, USEPA, Gulf Ecology Division; C.R. Jackson, J. Awkerman, USEPA. Determining the sensitivity of a diversity of species to environmental contaminants continues to be a significant challenge in

ecological risk assessment because toxicity data are generally limited to a few standard species. In many cases, QSAR models are used to estimate toxicity in the absence of measured toxicity values. We developed species sensitivity distributions (SSDs) and fifth percentile hazard levels (HC5) for aquatic species using three USEPA QSAR tools: ECOSAR (Ecological Structure Activity Relationships; [www.epa.gov/oppt/newchems/tools/21ecosar.htm](http://www.epa.gov/oppt/newchems/tools/21ecosar.htm)), ASTER (Assessment Tools for the Evaluation of Risk; [www.epa.gov/med/Prods\\_Pubs/aster.htm](http://www.epa.gov/med/Prods_Pubs/aster.htm)), and T.E.S.T. (Toxicity Estimation Software Tool; <http://www.epa.gov/nrmrl/std/cppb/qsar/>). QSAR estimates were used as input into the SSD module of the internet-based toxicity estimation program Web-ICE (<http://www.epa.gov/ceampubl/fchain/webice/>). In silico estimated HC5s were compared to HC5s developed from a dataset of over 14,000 toxicity records extracted from Ecotox and other sources. Preliminary results indicated that the input LC50 values generated by the three QSAR tools produced variable and often substantially higher estimates of acute toxicity than measured values. In general, chemicals with narcosis-based MOAs had the best agreement between measured and in silico estimated HC5s. Additional research will determine if in silico methods can be used to generate SSDs and accurate HC5s.

### 331 Embryotoxicity of Pulp Mill Effluent Extracts in Japanese Medaka

**(*Oryzias latipes*)** R. Orrego, Univ of Ontario Institute of Technology, Faculty of Science, Univ of Ontario Institute of Technology, post doctoral fellow; J. Guchardi, D. Holdway, Univ of Ontario Institute of Technology. Effects of Chilean pulp mill effluent extracts (solid phase extraction of untreated, primary and secondary treated pulp mill effluents), along with steroid standards (testosterone and 17 $\beta$ -estradiol) and a wood extractive standard (beta-sitosterol) were evaluated on developing post-fertilized medaka fish embryos. Our research has included a waterborne semi-chronic exposure experiment using 24hpf (unknown sex) d-rR (orange-red) mutant strain medaka embryos and two waterborne exposure experiments using 24hpf (unknown sex) FLFII (female leucophore free) and 72hpf FLFII (phenotypic sex-identified) mutant strain medaka embryos. Chronic exposure of both 24hpf d-rR and FLFII strain embryos showed similar delay in time to hatch and decreased hatchability with no significant egg and larvae mortality observed in all pulp mill extract exposed embryos. In contrast, significant early hatching and increased hatchability were observed in beta-sitosterol d-rR exposed embryos. High mortality was observed in all testosterone exposed embryos. Severe teratogenic responses were observed in medaka d-rR embryos in all treatments. Abnormalities included optical deformities (micro-opthalmia, 1 or 2 eyes) and lack of development of forebrains and hearts. Teratogenic responses such as axis malformation and pericardial edema were observed in all treatments using 24hpf FLFII with a high percentage related to males identified by fluorescent leucophore under microscope. Sex-related high mortality associated with severe malformations was observed in male and female embryos exposed to testosterone and pulp mill. Furthermore, signs of later masculinisation were observed in females exposed to testosterone where a slightly fluorescence of leucophores were detected before death. Differences in the severity of teratogenic effects seem to be related with the unique phenotype of each medaka mutant strains (d-rR and FLFII). Overall, our research has indicated that Chilean pulp and paper mill extractives caused medaka embryotoxicity (post-fertilized embryos) irrespective of the effluent treatment and different mutant strain used. The effects were mainly associated with delayed time to hatch, decreased hatchability, and sex-related teratogenesis and mortality.

### 332 Linking High-Throughput In Vitro Screens with EDSP Assays: The Casual Relationship Dilemma

E. Mihaich, Environmental and Regulatory Resources, LLC; C. Borgert, Applied Pharmacology and Toxicology, Inc.; L. Ortego, Bayer CropScience; B. Neal, Exponent; S. Gehen, Dow Agrosciences; W. Jones, CropLife America. One step in complying with Test Orders under the USEPA's Endocrine Disruptor Screening Program was compilation of Other Scientifically Relevant Information (OSRI). The extent and quality of OSRI was evaluated to determine what requirements were met by existing data, and hence, which of the 11 Tier 1 Endocrine Screening Battery (ESB) assays were needed. Many of the List 1 chemicals under ESB Test Orders were analyzed in the EPA's ToxCast Program, hence ToxCast data were submitted as OSRI in lieu of conducting many similar ESB in vitro assays, and to help support waivers for some in vivo assays. ToxCast seeks to predict human toxicity by measuring the activity of chemicals in a suite of some 500 cellular, biochemical and molecular assays conducted in high-throughput mode. The concept presumes that these assays can identify

a chemical's ability to activate so-called 'toxicity pathways.' In evaluating acceptability of OSRI, EPA rejected ToxCast results claiming that the reliability, responsiveness and relevance of ToxCast were undetermined and that ToxCast does not evaluate all known endocrine targets. In rejecting ToxCast data, EPA claimed its validation status was inferior to that of the individual ESB assays. However, the predictive value of the in vitro ESB assays for in vivo endocrine activity is also unknown at this time. Although EPA rejected ToxCast data as OSRI for the ESB, it used ToxCast data to assess and deem acceptable the risks of oil dispersants used for the Deep Water Horizon spill. These decisions have significant implications for the use of screening assays in regulatory programs, yet inconsistencies in application confound what those implications might be. Both the ESB and ToxCast programs appear to rely on correlative analyses rather than dispositive evidence that assays individually or in combination measure precursor events or frank toxicity. Clear and transparent criteria have yet to be defined for conducting weight of evidence evaluations of the ESB data. Although clearly defined biological activity is measured by most endpoints in the ESB, the 'toxicity pathways' ostensibly addressed by ToxCast assays are hypothetical. Despite potential advantages offered by ToxCast methods, it will be necessary to elucidate aspects of biological and physiological function as well as causal relationships between biochemical events and adverse effects before these data can offer improvements in the assessment of endocrine-mediated toxicity.

### 333 The DanTox-Project – Identification of Specific Toxicity and Molecular Modes of Action of Sediment-bound Pollutants in Zebrafish

H. Hollert, RWTH Aachen Univ, Institute for Environmental Research, Dept of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen Univ, Department of Ecosystem Analysis, RWTH Aachen Univ, Inst. for Environmental Research; S. Peddinghaus, J. Braunig, RWTH Univ; U. Feiler, 2German Federal Institute for Hydrology; c. Hafner, Hydrotex GmbH; M. Hammers-Wirtz, GALAC; N. Ho, 5Karlsruhe Institute of Technology; B. Kais, Aquatic Ecology and Toxicology Group; J. Otte, Karlsruhe Institute of Technology; R. Ottermann, RWTH Univ; S. Rastegar, Karlsruhe Institute of Technology; G. Reifferscheid, German Federal Institute for Hydrology; T. Braunbeck, Aquatic Ecology and Toxicology Group; U. Straehle, Karlsruhe Institute of Technology; S. Keiter, RWTH Univ. The aims of the DanTox project are a) to develop a suitable testing strategy for the assessment of bioavailable toxicants in sediments, b) to investigate the molecular and cellular mechanisms of sediment toxicity, and c) to elucidate the causality of biological effects. The long-term objective will be the development of a targeted cDNA-microarray which will be a useful tool for environmental screening. Sediment samples were collected from the Rhine River (Altrip and Ehrenbreitstein) and from the Vering Canal in Hamburg. For the testing of model chemicals methylmercury(II)-chloride, chlorpyrifos, Aroclor 1254 and bisphenol A were selected. The different methods of the project are categorized into four modules: a) bioassays – selected biomarkers and endpoints; b) gene expression – microarrays and qRT-PCR; c) data assessment – statistical evaluation of the data; d) practical transfer – comparison of the results from sediment contact tests and guideline tests. At the present stage of the project, results from the fish embryo test, EROD assay, live-imaging of EROD induction and qRT-PCR analysis are available. The highest embryotoxic potential was measured for the sample from Vering Canal after 48 h of exposure (EC50 = 2.6 and 3.6 mg/ml for extract and native sediments, respectively). EC50s for the extracts from Ehrenbreitstein (EC50 = 21.7 mg/ml) and Altrip (EC50 = 18.1 mg/ml) suggest that there is a comparable embryotoxic hazard potential. Only minor EROD induction was measured for TCDD suggesting that the barrier function of the chorion prevents TCDD from harming the embryo. In contrast, sediment extracts showed a clear dose-response-dependent EROD induction. Live-imaging of EROD induction with  $\beta$ -naphthoflavone as a positive control documented CYP1 induction at any time of inspection. Due to their molecular structure, methylmercury(II)chloride and chlorpyrifos showed no EROD induction. qRT-PCR revealed clear changes in the transcript abundance of CYP1A1, GST and UGT1A1 genes for the sediment extracts. Up to 600-fold changes in CYP1A1 could be seen for the extract from Vering Canal. First results from the biotest systems indicate that measurement of specific endpoints is a suitable strategy to identify and detect the bioavailable hazard potential of sediments. In addition, a comparison of the results from the EROD assay and qRT-PCR showed similar tendencies for the sediments indicating that the EROD assay with zebrafish could be a useful tool for routine testing.



**334 Transcriptomics and Transgenics – Novel Endpoints for the Fish**

**Embryo Toxicity Test (FET) to Infer Adverse Outcomes** M. Fenske, E. Muth-Koehne, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Dept of Ecotoxicology; V. Delov, RWTH Aachen Univ, Institute of Molecular Biotechnology (BioVII), Fraunhofer IME, Ecotoxicology; V. Schiller, RWTH Aachen Univ, Institute for Molecular Biotechnology (BioVII), Fraunhofer Institute for Molecular Biology and Applied Ecology, Dept of Ecotoxicology; A. Wichmann, RWTH Aachen Univ, Institute for Environmental Research (BioV); R. Kriehuber, Forschungszentrum Jülich GmbH, Dept of Safety and Radiation Protection; C. Schaefer, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Dept of Ecotoxicology. In aquatic ecotoxicology, the fish embryo toxicity test FET can be considered as one of the most promising animal alternative methods, and internationally considerable endeavors are being made to validate the FET as an alternative to the fish acute toxicity test (OECD203). In the US, zebrafish embryos are also used in the context of the ToxCast™ program of the EPA to screen for developmental toxicity. However, proof-of-principle studies have repeatedly shown that the FET can deliver more than just acute toxicity data, in particular when new and especially molecular endpoints are included in the test. Notably postgenomic approaches, like transcriptomics using microarrays, are well suited to evaluate subacute or teratogenic effects mechanistically, due to their high sensitivity and information rich data. They are also excellent tools for the identification of effect-specific markers, which prove very beneficial for (pre-)screening applications or for the prioritization of chemicals according to their hazard potential. We conducted gene expression studies with zebrafish and medaka embryos after exposure (for 48h and 7 days, respectively) to different chemicals. Effects of estrogenic and anti-androgenic compounds (genistein, BPA, linuron, prochloraz) were studied in both species; effects of insecticide were evaluated in zebrafish only. Functional analysis of regulated genes revealed that compounds suspected of endocrine activity in fish were able to disrupt endocrine system related pathways already in the embryos. Often, even the mode of endocrine disruption could be inferred from the transcriptome response. Insecticides like cartap or fenazaquin, affected the transcriptome very specifically, and the intersection of regulated genes was comparably larger for insecticides of similar than of dissimilar MOA. The use of GFP-expressing transgenic zebrafish lines is a novel and useful approach to enhance the mechanistic characterization of teratogenic effects in the FET. We use e.g., the transgenic line *Tg(fli1a:EGFP)*<sup>1</sup>, which expresses the enhanced green fluorescent protein in all blood vessels. This enables the assessment of vascular malformations at an early stage in the live embryo, and the effects are quantifiable, which is another advantage of this method over subjective morphological assessments.

**335 What Reductions in Fish Use Can be Made Employing Alternatives for Wastewater Effluent Assessment?**

P. Dorn, Shell Health, Ecotoxicology & Ecological Risk Ass; S.E. Belanger, The Procter & Gamble Company, Environmental Stewardship Organization, The Procter & Gamble Company, Central Product Safety Dept; T.J. Norberg-King, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; M.R. Embry, ILSI Health and Environmental Sciences Institute, ILSI Health & Environmental Sciences Institute (HESI), Senior Scientific Program Manager; P. Guiney, S.C. Johnson & Son, Inc., Global Safety, Regulatory & Environmental Assessment; M. Lampi, ExxonMobil Biomedical Sciences, Inc. Fish testing has many applications which may include new chemicals screening, product safety assessment, and water supply safety. The majority of fish testing use can be attributed to compliance and investigational testing of treated wastewaters throughout the world. Permitted wastewater discharges to surface waters are routinely tested in North America for effluent toxicity to determine compliance to state and national water quality standards. Similar mechanisms or goals exist in certain parts of Europe and Asia. Larval, juvenile and adult fish are employed for acute and/or chronic toxicity determinations. A major advantage of effluent toxicity testing is the integration of mixture toxicity and bioavailability. This is unlike chemical-specific analysis which identifies only individual constituents and can make it difficult to determine if receiving waters are protected. We suggest that a 3R approach for effluent testing will lead to adequate or new alternative solutions that are fit better designed for their purpose and enable fast, cost-effective equivalents. Alternatives for fish effluent testing include streamlining existing tests to use fewer fish, in vitro methodologies and fish embryo testing. We will review several options to reduce numbers of fish using techniques such as: combining control treatments, test designs using fewer effluent concentrations and possible development of fish

embryo tests yielding equivalent results to standard assays using swim-up/ and larval forms and chronic toxicity endpoints. A program has been initiated to test assumptions and assay conditions using fathead minnow and zebrafish. Comparisons of embryo tests and classical effluent survival and growth assays for both species are planned using chemicals and representative effluents. Successful results in the pilot experiment would prompt a more extensive program. Lastly, these assays may have additional utility to develop fish toxicity perspectives on complex mixtures and metabolites using benchtop wastewater treatment plant models (e.g., CAS units) during the development of new or existing chemical assessments.

**336 Neurochemical Biomarkers and Mercury Levels in Three Brain Regions of Wisconsin River Otters (*Lontra canadensis*)**

P.W. Dornbos, Univ of Michigan, Environmental Health Sciences; D. Nam, Univ of Michigan, Environmental Health Sciences; S. Strom, Wisconsin Dept of Natural Resources, Bureau of Wildlife Management; N. Basu, Univ of Michigan, Environmental Health Sciences, 1512 Plymouth Road Apartment 64. Fish-eating wildlife, such as River otters (*Lontra canadensis*), bioaccumulate concerning levels of mercury (Hg) which is a potent and ubiquitous environmental neurotoxicant. Despite such exposures, little is known about Hg-associated neurotoxic effects in this species and whether certain physiologically-important brain regions are particularly at risk. By studying river otter carcasses (N=40) from Wisconsin, we hypothesize that: A) brain total Hg levels will associate several neurochemical biomarkers, such as the N-methyl-D-aspartic acid receptor (NMDA) and benzodiazepine g-aminobutyric acid receptor (GABA<sub>A</sub>), as shown in previous investigations; and B) that associations will vary across three brain regions of physiological importance: Occipital cortex (Ox), Cerebellum (Cb), and the Brain Stem (Bs). In order to assess Hg exposure, a Direct Mercury Analyzer was used to measure total dry weight Hg concentrations within the three brain regions as well as fur, muscle, and liver. Mean Hg levels indicated the highest concentrations in Cb (0.95±0.76 ug/g), followed by Ox (0.89±0.69 ug/g), and lowest concentrations resided in Bs (0.66±0.36 ug/g). Statistical analysis indicated mean Hg concentration of Cb was significantly higher than Bs (P=0.037) while no significant differences were present among the remaining regions. Hg levels of fur (8.72±4.90 ug/g), muscle (1.98±1.35 ug/g), and liver (4.92±5.71 ug/g) were found to be significantly higher than the three brain regions (P < 0.01). Preliminary neurochemical assays (N=13) indicated no significant associations among total Hg and NMDA and GABA<sub>A</sub> receptor binding in the three brain regions. Additional samples and other biomarkers, such as glutamic acid decarboxylase and glutamine synthetase, will be analyzed to increase the power of this study. It is expected that these results will provide insight into the localization of Hg within the brain and the potential neurochemical effects associated with differing Hg concentrations within river otters and other mammalian wildlife.

**337 The Effects of Polychlorinated Biphenyls (PCBs) on Song Sparrow (*Melospiza melodia*) Songs**

S. DeLeon, R. Halitschke, A. Dhondt, Cornell Univ, Dept of Ecology and Evolutionary Biology. During the last century, polychlorinated biphenyls (PCBs) have emerged as harmful, worldwide chemical pollutants. Most research on the biological effects of PCBs on wildlife has emphasized the lethal effects of PCBs, such as embryo and juvenile mortality, and adult survival. However, it is well known that PCBs have long lasting developmental, reproductive and endocrine effects at sub-lethal exposures. The brain areas that produce song in birds are complex and highly vulnerable to both internal (i.e., developmental stress) and external (i.e., habitat fragmentation and parasitism) environmental stressors. Recent research shows that key areas in the learning and production pathways in the avian brain song system decrease in size when young birds are exposed to PCBs. Therefore, the goal of this research is to study consequences of sublethal exposures to PCBs on song production and quality in free-ranging song sparrows. During the breeding seasons (April-June) of 2006-2009 we studied Song Sparrows residing along the Hudson River, in northeastern New York. General Electric plants on the Hudson River discharged up to 1.3 million pounds of PCBs into the river between 1914 and 1977, resulting in the Hudson River being listed as a National Priorities List Superfund Site in 1984. Male Song Sparrow songs were recorded along the Hudson River, at locations above the point source pollution, and along a gradient downstream from the pollution source. Individual Song Sparrows were also captured at a subset of the recording locations in 2008 and 2009, and a whole blood sample and five feathers were collected from each individual along this PCB-gradient. PCBs were extracted according to established protocols after

addition of a stable isotope-labeled  $^{13}\text{C}_{12}$ -PCB mixture as an internal standard. A Solid Phase Extraction (SPE) cleanup produced the final extracts, which were analyzed by gas chromatography-mass spectrometry. We report quantitative PCB values from blood and feather samples, correlated with song data of males from the same regions, to test the hypothesis that there exists a direct link between PCB exposure and song in Song Sparrows.

**338 The Neurotoxic Potential of Emerging Perfluoroalkyl Acids (PFAAs) in Developing Chicken Embryos** C.G. Cassone, V. Vongphachan, Univ of Ottawa, Dept of Biology; S. Chiu, E. Pelletier, Environment Canada, Wildlife Toxicology; R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environment, Canadian Wildlife Service; C.L. Yauk, Health Canada, Environmental Health Centre, Carleton Univ, Dept of Biology; D. Crump, Environment Canada, National Wildlife Research Centre; S.W. Kennedy, Univ of Ottawa, Dept of Biology, University of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre. Perfluoroalkyl acids (PFAAs) are synthetic substances known for their chemical stability, resistance to degradation and potential to biomagnify in food chains. The toxicological and biological effects of PFAAs are not well characterized, although there is some evidence to suggest that they can impact neurodevelopment. Recent studies conducted in our laboratory have reported significant in vitro effects of two "replacement" PFAAs, perfluorohexane sulfonate (PFHxS) and perfluorohexanoic acid (PFHxA), on mRNA levels of thyroid hormone (TH)-responsive genes, including type II 5'-deiodinase (D2) and neurogranin (RC3), in chicken embryonic neuronal (CEN) cells. The present study attempts to confirm the in vitro effects of PFHxS and PFHxA in whole chicken embryos and to evaluate embryotoxicity, developmental endpoints and global gene expression profiles. PFHxS or PFHxA was injected into the air cell of 16-20 embryos per dose group at nominal concentrations of 0 (DMSO control), 10, 100, 1,000, 10,000 and 50,000 ng/g egg. Chicken embryos were incubated until pipping, at which point hatching parameters including pipping success, embryo and liver mass and tarsus length were recorded. Cerebral cortices were collected from eight embryos for chemical residue analysis and total RNA was extracted for subsequent real-time RT-PCR and microarray analysis. Pipping success decreased to 69% and 78% in the 50,000 ng PFHxS/g and 10,000 ng PFHxA/g dose groups, respectively. Embryo mass and tarsus length were significantly decreased, while liver somatic index was significantly increased in the 50,000 ng PFHxS/g dose group ( $n=16-20$ ,  $p<0.05$ ). No significant morphological changes were observed in the PFHxA-dosed embryos. Octamer motif binding factor 1 (OCT1), D2 and RC3 mRNA levels were significantly up-regulated in the cortex of embryos exposed to 10,000 and 50,000 ng PFHxS/g ( $n=4-8$ ,  $p<0.001$ ). D2 mRNA levels were significantly up-regulated in embryos exposed to 100 and 10,000 ng PFHxA/g ( $n=8$ ,  $p<0.05$ ). The present study successfully validates in vitro results concerning the modulation of key TH-responsive genes in an in vivo system and identifies adverse phenotypes in response to PFHxS treatment. Future microarray analysis of genome-wide expression patterns in developing chicken cortex will provide insight into the effects and mechanisms of action of PFHxS during neurodevelopment.

**339 Mercury Inhibits Brain Synaptosomal Glutamate Uptake Across Fish, Birds, and Mammals – Evidence from In Vitro, Laboratory, and Field Studies** D. Nam, Univ of Michigan, Dept of Environmental Health Sciences; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. Mercury (Hg) is a potent neurotoxicant that affects essential brain functions related to animal behavior and reproduction. While rodent studies have documented that a major mechanism underlying the neurotoxicity of Hg is inhibition of sodium-dependent transporters modulating glutamate uptake by astrocytes, such studies have not been studied in wildlife species. Here we explore the inhibitory effects of inorganic mercury ( $\text{HgCl}_2$ ) and organic mercury (MeHg) on glutamate uptake in brain synaptosomes from fish (seatrout, zebrafish, lemon shark, mako shark), birds (chicken, bald eagle), and mammals (mouse, mink, otter, seal). We use both in vitro and in vivo methods. Through in vitro screening assays, MeHg and  $\text{HgCl}_2$  inhibited the glutamate uptake in synaptosomes in a dose-dependent manner across species ( $\text{IC}_{50}$ : 0.39–2.3  $\mu\text{M}$  of  $\text{HgCl}_2$ ; 0.15–19.6  $\mu\text{M}$  of MeHg). Based on  $\text{IC}_{50}$  values, species sensitivities to glutamate uptake inhibition by  $\text{HgCl}_2$  can be generally ranked as: seatrout, zebrafish, mink ( $<0.5 \mu\text{M}$ ) > otter, mouse, lemon

shark, chicken ( $<2 \mu\text{M}$ ) > seal, bald eagle, mako shark ( $>2.0 \mu\text{M}$ ). Following in vitro MeHg exposure, seatrout ( $<0.5 \mu\text{M}$ ) was the most sensitive to glutamate uptake, followed by other 7 species ( $<2.0 \mu\text{M}$ ), and mink and seal (6.4–19.6  $\mu\text{M}$ ). Following in vivo studies on laboratory-exposed mink and chicken embryos and field-exposed bald eagles and lemon sharks, similar MeHg-related effects were observed. MeHg-exposed minks ( $n=12$ ; 2ppm MeHg in diet) had 40 times more MeHg in their brain and 35% less glutamate uptake compared to non-exposed controls ( $p<0.001$ ). Glutamate uptake was significantly reduced (30–35%) in chicken embryos exposed to MeHg (2, 3.2, 6.4  $\mu\text{g}$  MeHg/g egg) compared to control groups ( $n=11-18$  each;  $p<0.05$ ). Field studies on bald eagles also showed a negative correlation between brain total Hg (range: 0.29 – 13.5 ppm) and glutamate uptake ( $n=7$ ;  $r_p=-0.871$   $p=0.011$ ), while such a relationship was not observed in lemon sharks ( $n=27$ ;  $r_s=-0.327$ ;  $p=0.096$ ) probably due to relatively low brain Hg levels (0.05–0.34 ppm). Collectively, ecologically relevant levels of Hg can modulate glutamatergic pathways as demonstrated in series of in vitro, field work, and laboratory bioassay across fish, birds, and mammals. These comparative approaches may provide a framework for evaluating neurotoxic risks in the environment.

**340 Decreased Swimming Performance in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Associated to the Pyrethroid Pesticide Deltamethrin** A. Goulding, L. Shelley, Simon Fraser Univ; P.S. Ross, Fisheries and Oceans Canada, Institute of Ocean Sciences; C.J. Kennedy, Simon Fraser Univ, Dept of Biological Sciences. Deltamethrin and permethrin are neurotoxic pyrethroid insecticides commonly used both agriculturally and domestically, and have been identified as contaminants in sensitive aquatic environments. While pyrethroids are generally considered to present minimal risk of toxicity to terrestrial vertebrates, these chemicals have high lethal toxicity to fish. Except under rare conditions such as spills, the concentrations of pyrethroids in the environment are generally below levels expected to cause immediate lethality; however, these pesticides may lead to sub-lethal effects in exposed fish. Pesticide-associated changes to neuro-muscular function may translate into decreased swim performance and increased risk of predation, thereby increasing mortality rates and contributing to population-level declines. Since there are no readily available biochemical indicators of pyrethroid-induced neurotoxicity, swimming behaviour represents an integrated means of assessing toxicological injury in a manner that is ecologically relevant. Juvenile rainbow trout, *Oncorhynchus mykiss*, were exposed to either deltamethrin (0, 100, 200 or 300 ng/L) or permethrin (0, 0.5, 1, and 1.5  $\mu\text{g/L}$ ) for four days and subsequently assessed for swimming performance. Swim performance was reduced at the 200 and 300 ng/L doses of deltamethrin; however, permethrin did not affect swim performance at the concentrations tested and was therefore not investigated further. Subsequently, *O. mykiss* were exposed to deltamethrin (300 ng/L) for up to seven days and then allowed to recover in clean water, with assessment of swim performance on days 1, 4 and 7 of both exposure and recovery periods. Additional experiments will assess the ability of deltamethrin-exposed fish to avoid predation. This study suggests that sublethal exposures to deltamethrin leads to risks that are ecologically relevant, a finding that should be of value for regulators as they seek to protect fish and fish habitat from unintended impacts.

**341 Evolving Swimming Performance Endpoints in Fish for Water Quality/Toxicity Assessment** B. Berli, Univ of Basel, Man-Society-Environment Program; A. Ralph, L. Wiwchar, Univ of Alberta, Dept of Biological Sciences; P. Holm, Univ of Basel, Man-Society-Environment Program; K. Tierney, Univ of Alberta, Biological Sciences. Fish swim in waters increasingly impacted by human activities. Studies have found contaminants, temperature change, disease and flow rates can affect swimming performance. The majority of these studies have tested fish individually, in spite of many species tendency to school. We found the swimming performance of two juvenile salmonids, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), and adult zebrafish (*Danio rerio*) increased by as much as 2 fold when tested in schools. With acute exposure to low concentrations of calcium carbonate, a common constituent of sediment, rainbow trout swam considerably slower, but only when tested in schools. An interesting finding with zebrafish was that swimming performance became more variable with increased school size, and that a small proportion of fish consistently outperformed the school. Individual swimming performance is notoriously variable, but repeatable. We were able to associate some of the performance variation with increased mRNA and activity of two enzymes critical to aerobic and anaerobic metabolism (citrate synthase and



lactate dehydrogenase). We take three things from our experiments: testing fish in schools captures more realistic and greater performances; changes in swimming performance depend on whether fish are tested individually or in schools; and innate differences in enzyme activity may help explain performance variation. These findings will be useful in determining how environmental contaminants, such as the neuromodulatory pharmaceuticals we are currently testing, differentially impair the athletic phenotypes apparent in schooling fish.

### 342 Sub-network Enrichment Analysis Reveals Sexually Dimorphic Responses in the Largemouth Bass Hypothalamus After Dieldrin Exposure

C.J. Martyniuk, Univ of New Brunswick, Dept of Biology/Canadian Rivers Institute, Univ of New Brunswick, Biology; N.J. Doperalski, Univ of Florida, Dept of Physiological Sciences; K.J. Kroll, Univ of Florida, Physiological Sciences; D.S. Barber, Univ of Florida, Dept of Physiological Sciences; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. Organochlorine pesticides (OCPs) are a persistent class of environmental contaminant found in aquatic ecosystems worldwide. Studies demonstrate that the OCP dieldrin induces both neurotoxic and adverse reproductive effects in fish and mammals. The objective of this study was to characterize the effect of dieldrin in both female and male sexually regressed largemouth bass (*Micropterus salmoides*) (LMB) in a sub-chronic feeding exposure to 1) determine if the transcriptomic responses to dieldrin in the hypothalamus are conserved between the sexes and to 2) identify cell processes and signaling cascades underlying dieldrin neurotoxicity. Few studies address mechanisms of sexually dimorphic responses in the vertebrate CNS. Despite being a weak estrogen receptor agonist, we observed no adverse effects on reproductive endpoints that included GSI, plasma E2, and vitellogenin in males or females. Gene expression profiling and hierarchical cluster analysis revealed that the expression signature of dieldrin fed females and males grouped separately from control LMB but not from each other, suggesting that treatment influenced gene expression profiles more than the sex of the individual. However, male and female LMB showed marked differences in gene expression responses (only 11% transcripts were in common between the sexes). Functional enrichment analysis identified common biological processes and molecular functions affected by dieldrin that were related to steroid activity, stress, and protein folding (i.e., ubiquitin-proteasome pathway). Novel mechanistic insight was gained by performing sub-network enrichment analysis (SNEA), which revealed that in the female hypothalamus, dieldrin affected expression targets of GnRH, androgen, and GABA-A signaling whereas dieldrin affected expression targets of GnRH, somatostatin, and dopamine receptor signaling in males. This study demonstrates that there are sexually dimorphic responses to dieldrin in the vertebrate hypothalamus. This has implications for characterizing molecular responses and modes of action of aquatic pollutants in brain regions, as the neuroendocrine transcriptome of the sexes can respond quite differently at the genomic level to aquatic pollutants.

### 343 Using Magnetic Resonance Imaging to Determine the Effects of Thyroid Hormone Disruption on Brain Development

E. Montie, M. Powell, Univ of South Carolina Beaufort; T. Zoeller, Univ of Massachusetts Amherst; M. Parekh, T. Mareci, Univ of Florida. Humans, terrestrial wildlife, and aquatic animals are exposed to environmental chemicals that have been shown to interfere with the thyroid hormone system at various levels. This hormone system is especially important in white matter development. Volume data acquired from magnetic resonance images can be used as endpoints to assess white matter defects. A first step in this line of investigation is to determine how a goitrogen (i.e., a substance that suppresses the function of the thyroid gland) administered to pregnant rats affects brain volumes and white matter integrity in offspring. We exposed pregnant Sprague Dawley rat dams to propylthiouracil (PTU) from gestational day 6 until postnatal day (PD) 25 with 3 and 10 ppm PTU but continued the life of some offspring to P90. Exposure to 10 ppm PTU caused lowered levels of triiodothyronine (T3) and thyroxine (T4) in the serum of PD 25 pups, while the levels of P90 rats returned to normal. We performed 3D isotropic imaging *ex vivo* at P25 and P90 using a 750 MHz Wide Bore Spectrometer (17.6 T). Our preliminary analysis of these images shows a global loss of white matter that is visible at postnatal day 25. These early results suggest that MRI will provide a sensitive tool for the study of thyroid disrupting chemicals on brain development that can be used to quantify volume changes in white matter and potentially changes to the axonal structure of the brain.

### 344 Passive Sampling as Tool for Monitoring the Quality of Source Waters for Drinking Water Supplies

D. Alvarez, US Geological Survey, USGS Columbia Environmental Research Center; K. McCarthy, USGS; W. Cranor, USGS Columbia Environmental Research Center. Determining the quality of drinking water sources, especially with respect to anthropogenic organic chemicals, can be problematic using standard sampling techniques. Many contaminants, including emerging contaminants related to wastewater treatment discharges, are present at trace, but potentially biologically significant concentrations. The Eugene Water and Electric Board in Oregon has taken a proactive approach to monitoring for regulated and unregulated organic contaminants using the semipermeable membrane device (SPMD) and polar organic chemical integrative sampler (POCIS) passive samplers. The use of passive samplers for monitoring drinking water sources is a relatively untested concept in the United States. These samplers were deployed in 2007 and again in 2010 in source waters and the finished water targeting a suite of legacy and emerging organic contaminants. Few chemicals were identified, generally at concentrations in the subnanogram to nanogram per liter range. Among the chemicals commonly identified were several PAHs and the pesticides chlorpyrifos, dacthal, and the degradation products of endosulfan and DDT. Chemicals related to wastewater treatment discharges were generally not detected. A screen for estrogens and estrogen-mimicking chemicals, the yeast estrogen screen (YES), measured detectable levels of estrogenicity in tributaries to the McKenzie River; however, no estrogenicity was measured in the river itself. This pilot study demonstrated the ability to use SPMDs and POCIS to monitor the water quality of drinking water sources.

### 345 Integrated Water-Quality Assessment using Conventional Sampling, Passive Sampling, and Assay Techniques: Toward a More Holistic Understanding of Risk

K. McCarthy, V. Kelly, USGS; D. Alvarez, US Geological Survey, USGS Columbia Environmental Research Center. A combination of conventional point-in-time discrete water sampling, time-integrated passive sampling, and a bioassay are being used to assess the quality of the McKenzie River, which provides drinking water for nearly 200,000 water consumers in the vicinity of Eugene OR. The synergy of this three-pronged approach is allowing a more robust understanding than would be available from any one of the methods alone. Discrete water samples are being collected to provide information about how chemical concentrations vary with flow conditions (e.g., over the course of a storm). Semipermeable membrane device (SPMD) and polar organic chemical integrative sampler (POCIS) passive samplers are being used for time-integrated sampling and to allow measurements of chemicals present at concentrations below the limits of conventional point-in-time sampling and analysis techniques. Finally, the yeast estrogen screen (YES) is being used to provide insight into the biological effects of the mixture of compounds present in the water, including some that may not be targeted by the other two approaches. So far, during three separate sampling periods, we have detected 5 to 20 times more compounds with passive samplers than with conventional methods. In addition, positive results from the YES assay were associated with several potential endocrine disrupting compounds detected by passive samplers and indicate that estrogen activity is associated with urban land use in the basin. No compounds associated with endocrine disruption were detected using conventional sampling techniques.

### 346 USEPA/USGS Study of CECs in Source Water and Finished Drinking Water: Pharmaceuticals and Anthropogenic Indicator Compounds

E.T. Furlong, M. Noriega, US Geological Survey, National Water Quality Laboratory; S.T. Glassmeyer, USEPA, Office of Research and Development, USEPA, NERL/MCEARD/CERB; D. Kolpin, US Geological Survey. For more than 10 years, the presence and distribution of contaminants of emerging concern (CECs) in water supplies has been a subject of substantial scientific and public interest. The US Environmental Protection Agency and the US Geological Survey jointly designed and implemented a study of concentrations of CECs in source and finished drinking waters across the United States, sampling ground- and surface-water sources prior to and after treatment by various processes typically used to produce finished drinking water. The timing of collection of source and finished drinking water samples from each plant was adjusted by the hydraulic residence time in the plant to determine relative removal of CECs during treatment. An extensive quality assurance/quality control design was applied to this study to assess precision and accuracy of CEC determinations at ambient environmental concentrations. More than 185 pharmaceuticals and



anthropogenic indicator compounds were determined by high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) and by gas chromatography/mass spectrometry. Provisional results from a new direct aqueous injection LC/MS/MS method indicate that pharmaceuticals were detected in both untreated source water and finished drinking water samples, generally ranging in concentration from 2.5 to 400 ng/L. Precision at these ambient environmental concentrations was estimated from duplicate samples by calculating the relative percent difference (RPD). The median RPD ranged between 10 and 14 percent, with maximum RPDs of 74 and 83 percent in source and finished water, respectively. After correcting for ambient concentrations, median recovery of all pharmaceuticals from multiple matrix-spike experiments was 86 and mean recoveries of individual pharmaceuticals ranged between 59 and 116 percent. The relative standard deviations of spiked pharmaceuticals ranged between 7 and 22 percent. The median method detection limit (MDL) for 113 pharmaceuticals was 5.4 ng/L, with greater than 75 percent of individual compound MDLs less than 17 ng/L, indicating that method sensitivity is sufficient for evaluating pharmaceuticals in water sources used for domestic consumption.

**347 Pharmaceuticals and Endocrine Disrupting Compounds in Drinking Water: Occurrence, Treatment, and Transformation** M.J. Benotti, Battelle, Analytical and Environmental Chemistry. The presence of pharmaceuticals and endocrine disrupting compounds (EDCs) in drinking waters has generated a lot of discussion about what, if any, toxicological significance they pose. To compliment this ongoing discussion, this talk will present results from several studies to highlight 1) pharmaceutical and EDC occurrence in drinking water, 2) the efficacy of conventional and advanced forms of water treatment, and 3) novel approaches for elucidating the transformation products that are formed from some of these treatment technologies. Low nanogram-per liter concentrations of pharmaceuticals and EDCs are found in source and finished drinking waters throughout the United States, and the suites of compounds that are detected are a function of the type of chemical oxidation used during the treatment process. Advanced oxidation processes (AOPs) have been shown in pilot-scale studies or in water reuse applications to be particularly effective at reducing concentrations of these compounds and total estrogenic activity, though the energy costs incurred by these processes are very high compared to conventional processes. Scientists and regulators have recently begun to investigate the presence of transformation products of certain pharmaceuticals and EDCs. Identifying transformation products produced following chemical or photolytic oxidation is important given that they may also bear some toxicological significance. To illustrate this approach, the transformation of four compounds commonly detected in drinking water (atrazine, carbamazepine, diclofenac, and sulfamethoxazole) by low- and medium-pressure UV and UV-H<sub>2</sub>O<sub>2</sub> will be discussed. Experiments were conducted with a collimated beam apparatus and samples were analyzed by both LC-MS/MS and QToF-MS approaches. In each case, disappearance of the target analyte occurred concomitantly with the appearance of transformation products. The relative abundance of transformation products as a function of treatment can be used to hypothesize whether a transformation pathway is sequential (i.e., transformation products appear in a distinct sequence as a function of treatment) or non-sequential (i.e., transformation products appear with the same relative abundance as a function of treatment).

**348 Comparison of Toxicity Measures and Disinfection Byproduct Analyses for Evaluating Drinking Water Quality After Disinfection** B.A. Lyon, Univ of North Carolina at Chapel Hill, Environmental Sciences & Engineering; R.Y. Misk, H.S. Weinberg, Univ of North Carolina at Chapel Hill, Environmental Sciences and Engineering. As drinking water sources become increasingly impacted by anthropogenic activities, utilities are looking to alternative disinfection techniques to meet regulations and deliver quality drinking water to consumers. UV irradiation is one such process being used in some drinking water treatment plants as a primary disinfectant prior to terminal disinfection by chlorination or chloramination. It is important to understand the potential impacts of these processes and to evaluate the balance between microbial and chemical byproduct risk. Trihalomethanes (THMs) and haloacetic acids (HAAs) are two widely-occurring classes of disinfection byproducts (DBPs) whose quantity and speciation are sometimes used as indicators for formation of other DBPs. However, past research has demonstrated that while some alternative treatment processes may reduce the formation of THMs and HAAs, they can increase the formation of other DBPs which could potentially be of more concern to human

health. This paper demonstrates the value of measures other than THM and HAA formation as better representing DBP formation and overall finished water quality during UV treatment combined with chlorine or chloramines and includes both chemical and toxicity analyses. Water samples collected at the intake of a drinking water treatment plant were treated with chlorine or chloramine following UV irradiation in the presence and absence of nitrate, bromide and iodide, and compared to the same samples without UV but with a disinfectant dose adjusted for a similar target chlorine residual. Differences across treatments were investigated through analysis of total organic halogens (TOX) and TOX speciation (total organic chlorine, bromine and iodine), which was compared to individually measured DBPs (10 THMs, haloacetonitriles, halonitromethanes and haloketones). For toxicological assays, organic matter was concentrated by reverse osmosis (RO) and treated with different combinations of UV followed by chlorine/chloramine. RO concentration allowed for direct application of the disinfected samples to cells used for evaluating toxicity without the need for further treatment. Chronic cytotoxicity assays were performed using normal human colonocytes. This presentation will discuss the effects of UV treatment combined with chlorination/chloramination on the formation of DBPs, the overall incorporation of halogens into DBPs and indicate strategies for reducing drinking water toxicity associated with their presence.

**349 The Fate and Cellular Toxicity of Silver, Zinc Oxide, Titanium Dioxide Engineered Nanoparticles in Water** T.E. Abbott Chalew, Johns Hopkins Bloomberg School of Public Health, EHS; H. Huang, Johns Hopkins Bloomberg School of Public Health; K. Chen, Johns Hopkins Univ; K. Schwab, Johns Hopkins Bloomberg School of Public Health. Engineered nanoparticles (NP) widely used in consumer products may end up in the aquatic environment with potential impacts on aquatic organisms and humans. This project investigated NP stability in synthetic surface waters and the toxicity of aqueous NPs to human gut cell lines. We purchased unmodified, commercially available nanoparticles including silver (Ag), titanium dioxide (TiO<sub>2</sub>), and zinc oxide (ZnO) with similar sizes as specified by the manufacturers. Average particle sizes measured by transmission electron microscopy were 34 nm, 36 nm, and 84 nm for TiO<sub>2</sub>, ZnO, and Ag, respectively. Over 90% of particles were aggregated in ultrapure water. NP size was monitored over 48 hours in synthetic freshwaters (SFW) without and with natural organic matter (SFWN) (n=12 for both) using dynamic light scattering. After 48 hours, NPs in SFWN were the smallest (248 nm, 287 nm, and 201 nm for TiO<sub>2</sub>, ZnO, and Ag) compared to SFW (4970 nm, 9050 nm, and 255 nm for TiO<sub>2</sub>, ZnO, and Ag). Human gut epithelial Caco-2 cells were exposed to NPs in media from 0-1000 ppm for 24 and 48 hours. Cell viability and cell stress are being evaluated. Significant cell death was observed at 50 ppm ZnO NPs (60%). No significant death in TiO<sub>2</sub> or Ag exposed cells was observed. NPs in SFWN are less than 300 nm and remain in suspension, which suggests they will persist in surface and drinking waters. Therefore, it is critical to understand the effects of ingested nanoparticles to protect human health.

**350 Presence of Arsenic in the Environment in Southwestern USA and Northern Mexico** L.M. Camacho, Univ of Texas at El Paso, Center for Inland Desalination Systems; M. Gutierrez, Missouri State Univ, Dept of Geography; M.T. Alarcon-Herrera, Advanced Materials Research Center; M.d. Villalba, Chihuahua Autonoma Univ; S. Deng, New Mexico State University, Chemical engineering Dept. The presence of arsenic in the arid region of northern Mexico (states of Chihuahua and Coahuila) and the bordering states of the southwestern US (New Mexico, Arizona, and western Texas) were reviewed in this article. Main sources of arsenic in the study area were found to be primarily of natural origin. However, sources of non-naturally occurring arsenic included pesticides, sewages and smelting plants. Potential future Arsenic release from sediments of the Rio Concho Basin in northern Mexico into the water column is a cause of concern. Exposure to arsenic contamination has shown to be detrimental to the central nervous system and for cognitive development in children, independent of socio-demographic variables, nutritional status, and levels of blood lead. Even though a variety of methods to treat contaminated water and soil are available, in the area of study, enhanced and emerging technologies have been applied in most of the cases only at laboratory or bench scale. Phytoremediation stands out as a promising technology for removal or arsenic in this region.

**351 Arsenic Adsorption Influences Safe Drinking Water Options in Bangladesh**

**K.A. Radloff**, LDEO, Columbia Univ; Y. Zheng, LDEO, Columbia Univ, Queens College, CUNY; H.A. Michael, Univ of Delaware; M. Stute, LDEO, Columbia Univ, Barnard College; K. Ahmed, Dhaka Univ; A. van Geen, LDEO, Columbia Univ. An estimated 100 million people are exposed to elevated levels of As by drinking untreated groundwater tapped by manually operated tube wells in South Asia. In Bangladesh, the high spatial variability of dissolved As in the shallow aquifer makes predicting the location of safe, low-As groundwater difficult. The same spatial variability, however, also creates an opportunity for reducing As exposure by targeting low-As wells. A major question remains: To what extent could low-As wells be contaminated by the intrusion of high-As groundwater, especially in areas with extensive municipal and agricultural pumping? The purpose of this study was to quantify the extent to which retardation and adsorption can protect low-As aquifers from contamination. Two field sites were chosen to investigate arsenic adsorption in a shallow and a deeper aquifer. At the shallow aquifer site, groundwater As concentrations increased from 30 L kg<sup>-1</sup>. The push-pull tests confirmed the differences between the shallow and deeper sands, however partitioning estimates were generally lower. The results of these experiments provide further evidence that groundwater As concentrations are controlled by As sorption on sediment and sorption reaches equilibrium rapidly compared to the time scale of groundwater flow. The implication of this work is that groundwater As concentrations in both shallow and aquifer are currently stable, as mobility is retarded by adsorption, but remain vulnerable as groundwater flow conditions are perturbed at a growing scale.

**352 Bioaccumulation of Biosolids-borne Triclosan (TCS) in Terrestrial Organisms**

**M. Waria**, Univ of Florida, Soil and Water Science Dept, Univ of Florida, Soil and Water Science; G.A. O'Connor, G.S. Toor, Univ of Florida, Soil and Water Science; C. Wilson, Univ of Florida. Triclosan (TCS) is a common antimicrobial agent found in many personal care products and subsequently detected in wastewater treatment effluents and biosolids. Land application of biosolids can transfer significant quantities of TCS to the terrestrial environment. Our objective was to quantify the accumulation of biosolids-borne TCS in multiple food crops and earthworms. A recent greenhouse study found significant TCS accumulation [bioconcentration factor (BCF) = 3 to 6.5] in plant parts of a single crop (soybean) grown in soil amended with TCS spiked liquid biosolids. However, accumulation can vary with the plant species and type of biosolids (liquid vs cake) utilized. Our study evaluated plant accumulation in lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), and bahia grass (*Paspalum notatum*), representing monocotyledons (monocots), dicotyledons (dicots), above-ground (leaves), and below-ground (roots) biomass. We grew plants in a field collected soil that was amended with cake biosolids at a high application rate (228 Mg ha<sup>-1</sup>) once and field equilibrated for 2 years. The resulting TCS concentration was ~1 mg TCS kg<sup>-1</sup> amended soil. The plants were either grown under growth chamber or greenhouse conditions. The TCS concentration utilized in the study did not adversely affect crop growth (dry matter yields). Accumulation was negligible in radish [bioaccumulation factor (BAF) = < 0.001] and bahia grass leaves (BAF = < 0.001), minimal in lettuce leaves (BAF = 0.01), but greater in radish roots (BAF = 0.10). Dicots (radish, lettuce) accumulated more TCS than the monocot (bahia grass). The BAF values suggest some accumulation in below-ground biomass (roots), but minimal translocation to above-ground biomass (leaves). We also collected earthworms from the same biosolids amended field soil. The average TCS concentration measured in the earthworms was 4.3 ± 1.9 mg kg<sup>-1</sup> corresponding to an average bioaccumulation factor (BAF) of 4.3 ± 0.7. Preliminary risk assessments suggest that the pathways of plant consumption by human, animals; and soil consumption by earthworms present minimal risk from biosolids-borne TCS.

**353 Bioaccumulation of Perfluorochemicals in Earthworms**

**C. Rich**, A. Blaine, Colorado School of Mines; C. Higgins, Colorado School of Mines, Environmental Science and Engineering. Perfluorochemicals (PFCs) are found in a variety of household products used every day including everything from stain repellents to non-stick pans. Biosolids derived from wastewater treatment plants are commonly land-applied in the United States, and as a result, biosolids-amended soils often contain high levels of PFCs. Recently, concerns have arisen about potential impacts to soil ecosystems following the land application of biosolids. Specifically, bioaccumulation of PFCs in the earthworm is of great interest since it represents a direct link between the soil and higher trophic levels. Previous studies have

found that PFCs from sediment can bioaccumulate in aquatic worms. This study examined PFC bioaccumulation in earthworms living in biosolids-amended soils using a standard 28-day exposure. Two different field soils amended with high and low biosolids loading rates were used. Results from this study have important implications with respect to the potential transfer of PFCs from biosolids into the terrestrial food chain.

**354 Uptake and Transformation of Estrogenic Compounds by Corn**

**M. Card**, The Ohio State Univ, School of Earth Sciences; **Y. Chin**, The Ohio State Univ, Dept of Geological Sciences. Hormonally active compounds are emerging contaminants of concern, and runoff from manure-fertilized fields represents a major, uncontrolled source of natural and synthetic estrogens in the environment. Uptake and transformation by crop plants and associated microbes may significantly limit the environmental mobility and effects of manure-borne estrogens. We exposed maize (corn) seedlings to hydroponic solutions containing one natural estrogen (17β-estradiol [17β-E2] and estrone [E1]) or a synthetic estrogen mimic (zeranol [α-ZAL] and zearalanone [ZAN]). At given time points, four replicates of each treatment, including estrogen-free blank controls and plant-free glassware controls, were collected and destructively sampled. Plant tissues were homogenized and extracted in methanol, and analyte concentrations in the aqueous phase and methanol extracts were quantified by reverse-phase high pressure liquid chromatography (RP-HPLC). Transformation products were qualitatively identified by gas chromatography with mass spectrometry (GC-MS). After 22 d of exposure to maize seedlings, aqueous α-ZAL concentrations decreased 99% and ZAN concentrations decreased 96%. 17β-E2 and E1 were undetectable in the aqueous phase after 12 and 18 d of exposure, respectively. The seedlings performed reversible transformations between 17β-E2 and E1 and between α-ZAL and ZAN, whereas the plant-associated microbes only completed the oxidation step. Taking into account the relative estrogenicity of these natural and synthetic hormones, and the rate at which each was produced as transformation products, the observed transformations generally led to an overall reduction in the estrogenicity of the solutions. Further, exposure to these estrogens did not elicit significant changes in germination, growth, or enzyme activities of maize seeds. Although significant increases in all of these measures have been previously reported in corn exposed to 17β-E2, we did not observe changes in germination rates; root and shoot lengths; or activities of amylase, peroxidase, polyphenol oxidase, or catalase. Taken together, these results indicate that maize seedlings may have a significant impact on the environmental fate of manure-borne natural and synthetic estrogens, while exposure to these hormones likely has no detrimental effect on the plants.

**355 In Situ Aquatic Flame-retardant Exposure and Trophic Transfer**

**M.J. La Guardia**, Virginia Institute of Marine Science, Environmental & Aquatic Animal Health, Virginia Institute of Marine Science, Environmental Science; R.C. Hale, E. Harvey, Virginia Institute of Marine Science, Environmental & Aquatic Animal Health. Based on concerns over potential health effects caused by some flame-retardants (FRs), two of the three-polybrominated diphenyl ether (PBDE) formulations (Penta- and Octa-BDE) were recently added to the Stockholm Convention's Persistent Organic Pollutants (POPs) list (Stockholm Convention, May 2009) and their usage and manufacture restricted. The third formulation, Deca-BDE is now banned in the EU and will be phased out of production in the U.S by the end of 2013. An additional FR, hexabromocyclododecane (HBCD) is also classified as persistent and bioaccumulative. It may cause reproductive developmental and neurological disorders. HBCD will be phased-out of European commerce by 2015. In the US, a USEPA review of HBCD is due by the end of 2011. To meet fire safety standards, consumer products still need to contain FRs. However, the environmental fate of several PBDE replacement products (i.e., 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH) and 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE)) is largely unknown. We observed TBB, TBPH and BTBPE in sediments and to bioaccumulate in benthic organisms (*Corbicula fluminea* and *Elmimia proxima*) collected downstream from a US textile manufacturer's outfall. The summed totaled body burden of these replacement products ranged from 152- to 2230 ng g<sup>-1</sup> lipid weight (lw). These mollusks also exhibited ΣPBDEs 64,900 and 47,200 ng g<sup>-1</sup> lw, and ΣHBCDs 363,000 and 151,000 ng g<sup>-1</sup> lw, respectively. To further identify the aquatic fate of these alternative-FRs, critically needed for risk management evaluation, additional samples were collected at the aforementioned textile outfall. These included benthic predators and higher trophic

vertebrates. Samples were analyzed by liquid chromatography-atmospheric pressure photoionization (APPI) tandem mass spectrometry for  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD, TBB, TBPH and BTBPE including FRs routinely analyzed by gas chromatography mass spectrometry (i.e., PBDEs). Details of this in situ field study will be discussed including the aquatic trophodynamics of multiple FR exposure. Results using this novel ionization approach for FR analysis will also be introduced. A comparative analysis between the bioavailability of PBDEs, HBCD and alternative-FRs and their trophic transfer will be presented.

**356 Bioaccumulation of Legacy POPs and Emerging Contaminants in Tropical Mangrove Food Webs** B.C. Kelly, S. Bayen, H. Zhang, Y. Teo, National Univ of Singapore, Dept of Civil and Environmental Engineering. Tropical mangroves are important ecosystems in Southeast Asia, as well as other tropical and subtropical regions of the world. These unique ecosystems provide essential habitat for numerous species of aquatic and terrestrial organisms. Previous studies have demonstrated the occurrence and bioaccumulation of legacy persistent organic pollutants (POPs) such as PCBs, DDTs and PBDEs in Singapore mangroves. For example, SPCB concentrations were approximately 1 ng/g dry weight in sediments, 7 ng/L in sub-surface waters and between 0.5 to 100 ng/g wet weight in biota. The objectives of the present study are twofold: (i) conduct a field study to assess the occurrence, levels and bioaccumulation behavior of several emerging contaminants of concern in Singapore mangrove ecosystems and (ii) develop and evaluate a mechanistic model representing the bioaccumulation kinetics and trophodynamics of organic contaminants in tropical mangrove food webs. The field survey focused on detection of pharmaceuticals and personal care products (PPCPs), synthetic musks and current-use brominated flame retardants (BFRs) in samples of water, sediments and mangrove organisms (algae, zooplankton, worms, mollusks, crabs and fish) from two local mangroves. Following standard extraction and cleanup methods, identification and quantification of target compounds was performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) or gas chromatography triple-quadrupole tandem mass spectrometry (GC-MS/MS). Observed concentrations and bioaccumulation potential of target analytes were compiled and compared to those of legacy POPs. We further employed a mechanistic bioaccumulation model to predict steady-state chemical concentrations, species-specific bioaccumulation factors (BAFs) as well as trophic magnification factors (TMFs) based on ambient concentrations (air, water, sediments) and physical-chemical properties. The results indicate that chemical KOW greatly influences the chemical bioaccumulation in aquatic organisms such as zooplankton and fish, while chemical KOA is shown to be a key determinant for chemical biomagnification in air-breathing organisms inhabiting the mudflats and terrestrial zones. The model also highlights the significance of metabolic transformation rate, which undoubtedly plays a major role in the bioaccumulation potential of organic chemicals in the food webs.

**357 Occurrence, Fate, and Biological Consequences of Antidepressants in Fish Living in Effluent-impacted Streams** M.M. Schultz, College of Wooster, Dept of Chemistry; E.A. Sakach, The College of Wooster, Biochemistry and Molecular Biology; E.T. Furlong, US Geological Survey, National Water Quality Laboratory; D. Kolpin, P. Phillips, US Geological Survey; N. Chavez, The College of Wooster, Dept of Chemistry; H. Schoenfuss, St. Cloud State Univ, Aquatic Toxicology Laboratory, St. Cloud State Univ, Dept of Biological Sciences MS-273. Antidepressants are widely prescribed pharmaceuticals in the United States. As conventional treatment has limited removal of many pharmaceuticals, municipal wastewater treatment plants have been found to be an important source of antidepressants to the environment. Thus, fish residing in the effluent-impacted streams are receiving continuous exposure to antidepressants, causing a potential concern to non-target organisms. A liquid chromatography/tandem mass spectrometry method was applied to measure antidepressants in aqueous samples and fish tissues from field and laboratory studies. A field study (2010) to measure antidepressants in brain tissues from native white suckers residing in effluent-impacted streams was conducted at points upstream and at points progressively downstream of select effluent outfalls in New York State (NY). Antidepressant profiles observed in the brains from this study substantially differed from the profiles previously observed at other geographic locations. Duloxetine was present at higher concentrations in the fish brains in the 2010 study as compared to the levels observed in previous studies conducted in 2005 and 2006, perhaps reflecting the increase in prescriptions

dispensed over that time period. In addition, this NY field study reinforces the relation between effluent concentrations and corresponding fish brain concentrations. For example, a NY site with orders of magnitude higher effluent concentrations of bupropion than observed in previous studies also had parallel orders of magnitude higher fish brain bupropion concentrations. Complementary laboratory exposure studies were conducted on adult fathead minnows (*Pimephales promelas*) and adult bluegill (*Lepomis macrochirus*). Results from both laboratory exposures suggest that there is preferential uptake of select antidepressants, and that fish can metabolize fluoxetine to its biologically active metabolite, norfluoxetine, which, similarly to parent fluoxetine, can inhibit the presynaptic reuptake of serotonin.

**358 The Role of Periphyton in the Removal of Trace Organic Compounds in an Engineered Treatment Wetland** J.T. Jasper, Univ of California – Berkeley, Dept of Civil and Environmental Engineering; D.L. Sedlak, Univ of California – Berkeley, Dept of Civil & Environmental Engineering. Engineered treatment wetlands offer a cost-effective means of removing trace organic contaminants from municipal wastewater. Treatment wetlands designed to remove nutrients often employ dense vegetation and reducing condition to enhance rates of denitrification and particle settling. However, these systems are often inefficient and could actually accumulate organic contaminants. To enhance rates of removal of trace organic contaminants, a treatment system was designed with shallow water and a coating of periphyton on the bottom. This configuration yields enhanced rates of photolysis, opportunities for contaminant removal through sorption and contaminant transformation by a diverse microbial community. To better understand the role of periphyton in an unvegetated engineered treatment wetland, the removal efficiency of six compounds was studied in a pilot-scale system and under well-controlled laboratory conditions. All compounds had half-lives of less than two days in the aqueous phase, except carbamazepine, which behaved conservatively. Sorption to periphyton coupled with biotransformation was an important removal mechanism for compounds that were not amenable to photolysis, such as atenolol, metoprolol, and trimethoprim. Photolysis was important for the compounds propranolol and sulfamethoxazole and was enhanced at elevated pH values, caused by periphyton photosynthesis. Measurements of reactive oxygen species and other transients generated by photolysis indicated that indirect photolysis in the sunlit treatment wetland is attributable to the simultaneous production of several different oxidants.

**359 Background PAHs in Urban Watersheds, Beneficial Use of Dredged Material, and the Case for Relative Risk Assessment for Urban Environment Restoration** J.P. Kreitinger, AECOM Environment, US Army Engineer Research and Development Center, CEERD-EP-R; R.A. Price, A.J. Kennedy, US Army Engineer Research and Development Center. The US Army Corps of Engineers is responsible for the maintenance of 58 federal navigation channels in the Great Lakes. To maintain Cleveland, OH harbor, approximately 250,000 cubic yards of sediment must be dredged and managed each year. Complicating the management of dredged material is that most of the sediment is considered 'contaminated' and is managed by placement in confined disposal facilities (CDFs) constructed along the Lake Erie waterfront. The capacity in waterfront CDFs is limited and a comprehensive review of the suitability of the dredged material for various beneficial uses has been conducted. Sediment in the Cleveland Harbor navigation channel was determined that have Total PAHs (sum of 13) ranging in concentration from 8 to 21 mg/kg exceeding the consensus threshold effect concentration (TEC) but below the probable effect concentrations (PEC) considered to be protective of aquatic life. No toxicity was observed in sediment having 21 mg/kg Total PAHs using the aquatic invertebrates *Hyalella azteca* and *Chironomus dilutus* nor the terrestrial worm *Lumbriculus variegatus*. Characterization of the potential narcotic toxicity resulting from PAHs using ASTM Method D7363-07 demonstrated low PAH bioavailability and little contribution to narcotic toxicity (< 0.15 Toxic Units). Sediment previously dredged from the navigation channel from 1979 to 1999 (having similar or higher concentrations of PAHs) is currently managed as an 88-acre urban nature preserve that has numerous native plant and animal species. In contrast, recent data has been reported on the concentration of PAHs in an urban Cleveland neighborhood consisting of over 100 abandoned houses that is being considered for urban land rehabilitation. The average concentration of benzo[a]pyrene, the primary risk driver for human health, in neighborhood soils is approximately twice the average concentration observed in the navigation channel sediment. These data demonstrate



that the risk to human health from beneficial use of dredged material for urban land restoration would be approximately 1/2 the risk that results from exposure to existing soil in some urban neighborhoods. From a public health and ecological perspective the beneficial use of dredged material as soil in Cleveland's highly urbanized environments could reduce exposure to PAHs. The assessment of relative risk must be considered for successful restoration of human and ecological habitat in urban environments.

**360 Developing Menhaden as a Model Organism of Studying the Deep Water Horizon Oil Spill** C. Bentivegna, Seton Hall Univ, Dept of Biological Sciences; J. Sowa, N. Snow, Seton Hall Univ, Dept of Chemistry/Biochemistry; K. Cooper, Rutgers, the State Univ of New Jersey; R. Portier, Louisiana State Univ. The Deep Water Horizon (DWH) oil spill is likely to have widespread and chronic effects on the Gulf of Mexico's ecosystems and fisheries. The release of petroleum from the BP's Macondo 252 well started on April 20, 2010 and continued for 87 days until the well was capped on August 3, 2010. A major goal of this research project was to determine the levels and types of polycyclic aromatic hydrocarbons (PAH) found in a common filter-feeding fish called menhaden. Menhaden is an oily, prey fish used commercially to produce omega-3. Due to their oily nature, it was anticipated that menhaden would accumulate oil contaminants and potentially transfer them to predatory fish. For this project, adult menhaden (28-35 cm) were collected in 2010 from the James River, VA, Delaware Bay, NJ and Grand Isle, LA. Grand Isle received heavy oiling of its shores and wetlands from the DWH spill. It was anticipated that collecting menhaden from VA and NJ as well as LA would allow discrimination of a BP oil spill signature from that of general urbanization. Studies have included 1) analyzing PAHs in DWH source oil and fish oil by headspace solid-phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS), 2) analyzing fish liver for PAH metabolites using scanning fluorescence spectroscopy, 3) conducting trophic transfer studies by exposing mummichog and bluefish to fish oil by gavage and 4) conducting histopathology on "peanut" menhaden collected from the same sites. Preliminary data has indicated that HS-SPME GC-MS can detect PAHs in fish oil at part per billion levels and that LA fish show higher levels of naphthalene-like PAHs but lower levels of pyroxyrene-like PAHs than VA and NJ fish. There also were characteristic patterns of naphthalene-like PAH accumulation. This project should help develop menhaden as a model organism for monitoring oil spills.

**361 A Multi-Site Model to Estimate the Toxicity of PAH-Contaminated Sediments at MGP Sites** M. Kierski, Exponent, Ecological and Biological Services Practice; A. Morrison, Exponent, Inc., EcoSciences; S. Kane Driscoll, Exponent Inc.; C. Menzie, Exponent, Inc., Exponent, Principal. Many manufactured gas plant (MGP) sites have left a legacy of polycyclic aromatic hydrocarbon (PAH)-contaminated sediments in adjacent waterways. At three MGP sites in the Midwest, the toxicity of PAH-contaminated sediments to benthic invertebrates was evaluated using 1) laboratory-based sediment toxicity test results for *Hyalella azteca*, and 2) predictions of toxicity using the US Environmental Protection Agency's equilibrium partitioning sediment benchmark (ESB) methodology. Site-specific toxicity-based concentration thresholds for total PAHs (based on 13 analytes) varied considerably among the three sites (i.e., 103 mg/kg to 712 mg/kg total PAHs). To evaluate whether the differences in these toxicity-based threshold concentrations was a function of sediment characteristics, the data for all three sites were analyzed collectively. Total PAH concentrations were normalized based on total organic carbon (TOC) content, black carbon content, a combination of TOC and black carbon, or with the ESB methodology to evaluate the consistency of PAH thresholds. Receiver operating characteristic (ROC) curve analysis was used to determine whether each indicator (e.g., TOC-normalized total 13 PAH concentrations) was a good predictor of sediment toxicity and to develop multi-site thresholds based on toxicity tests and ESB values. All indicators investigated were good predictors of survival. Very few adverse impacts on growth were observed. Thresholds estimated on the basis of concentrations of total 13-PAH or total 34-PAH (and the normalized equivalents) were equally good indicators of toxicity. Thresholds predicted using the multi-site data set were compared with thresholds calculated for individual sites. This modeling effort demonstrated that ROC analysis of normalized data from multiple sites can be used to develop sediment toxicity thresholds that are often higher than traditional sediment quality benchmarks, but still are protective of aquatic organisms.

**362 PAH Distribution and Source Association in Sediments and Mussel Tissue the NH Great Bay Estuary** A. Watts, S. Jones, Univ of New Hampshire. Polycyclic aromatic hydrocarbon concentrations have been monitored in sediment and mussel tissue in the NH Great Bay Estuary through the EPA National Coastal Condition Assessment program and the Gulfwatch program. Samples have been collected at 154 sites in the Great Bay and in Rye and Hampton harbors, from 2000 to 2006. PAH concentrations in mussel tissue in portions of the Great Bay have been increasing gradually since 1993. Spikes in concentration appear to be correlated with oil spills and dredging activities, the latter probably reflecting re-suspension of legacy contaminants, but all factors contributing to the trend have not been definitively identified. Potential sources include increased urban watershed runoff, atmospheric deposition, and sediment erosion. Characteristic compound patterns can be used to identify source categories, and source receptor models can be used to identify dominant sources of PAHs in sediments. Initial analysis of sediments samples collected in sections of the estuary are consistent with coal tar, and are probably associated with dredging or other disturbances in the vicinity of a former manufactured gas plant. Concentration distributions in sediments and tissue with respect to known and suspected source events will be discussed, and a more detailed source receptor model will be performed to determine if specific watershed characteristics and land use patterns are associated with elevated PAHs in sediment and tissue.

**363 Genetic and Epigenetic Mechanisms of Environmental Injury by Benzo[a]pyrene and Related Polycyclic Aromatic Hydrocarbons** K.S. Ramos, Univ of Louisville, Biochemistry and Molecular Biology. This presentation will highlight recent findings implicating L1 (Long Interspersed Nuclear Element-1) in the regulation of renal morphogenesis. Retroelements such as L1 are silenced epigenetically in terminally differentiated cells, and become highly active in embryonic, undifferentiated, and transformed cells. Using the mK4 murine embryonic kidney cell model we recently showed that somatic cells harbor retrotransposition events upon ectopic expression of L1RP (L1 from Retinitis Pigmentosa) and that overexpression of L1RP modulates differentiation programming in these cells through a feedback loop that involves L1, WT1 and AHR. These genetic alterations are recapitulated by exogenous activation of L1 by polycyclic benzo[a]pyrene and dioxin, suggesting that L1 represents a key molecular target in the modulation of genetic and epigenetic programs by environmental chemicals.

**364 Proposed Increases in PAH Relative Potency Factors Will Greatly Increase Risks at All PAH Sites** B. Magee, D. Chin, ARCADIS. The United States Protection Agency (USEPA) is proposing to modify the approach that it has been using since 1993 to evaluate polycyclic aromatic hydrocarbon (PAH) mixtures. While USEPA previously considered seven PAHs to have carcinogenic potential, the proposed approach increases to 26 the number of PAHs considered to be carcinogenic. In addition, according to the proposed approach, a number of the PAHs are being assigned a Relative Potency Factor (RPF) that is greater than 1 relative to benzo[a]pyrene. Several proposed RPFs are greater than 10 including benzo[c]fluorene (20), dibenzo[a,h]pyrene (30), and benz[j]aceanthrylene (60). If this approach is adopted, it will be necessary to quantify all 26 compounds in media at contaminated sites. Many of these additional constituents are not included in any standard analytical methods. In addition, it may be necessary to re-open and reassess sites that have already received regulatory closure. However, there are many scientific criticisms of the USEPA proposal. Most importantly, USEPA has not performed a Weight of Evidence Evaluation as called for in its Guidelines for Carcinogen Risk Assessment (EPA, 2005). Second, USEPA assumed that all PAHs act by a similar mode of action with no supporting evidence. USEPA also failed to validate the derived RPFs using cancer response data from real world complex mixtures. As noted in Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures (USEPA, 2000), data from whole mixtures are preferable to data from mixture components. We have performed several validation exercises that demonstrate that USEPA's RPFs overestimate the true carcinogenic risk observed when the interactions between components are inherently taken into account in mixture studies. Lastly, there were many technical problems associated with specific RPFs. For instance, many RPFs are based on a single study or "low confidence" studies; some RPFs are based on in vitro assays; some RPFs are derived from studies in which the chemical identity of the test substance was not confirmed; some RPFs are based on studies with unusual modes of administration, such as lung implantation in unusual

matrices; and many studies exceeded the Maximum Tolerated Dose. This presentation discusses the implications for human health risk assessment and presents key scientific criticisms presented in our recent comments to USEPA on the new guidelines.

**365 Risk Evaluation of Polycyclic Aromatic Hydrocarbon Mixtures** C. Liu, B. Krupka, CDM; C. Julias, CDM, CDM, Chemical Engineer; N. Luke, CDM. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic chemicals that persist in the environment. PAHs are formed through an incomplete combustion of most organic material and also occur naturally at low levels of crude oil and coal. Currently, there are seven PAHs considered by the USEPA as probable human carcinogens (cPAHs) by a mutagenic mode of actions. Toxicity criteria are currently available only for benzo[a]pyrene. The remaining cPAHs are assessed using PAH-specific relative potency factors (RPFs) that express the potency of these cPAHs relative to benzo[a]pyrene. The California EPA (Cal/EPA) considers 24 PAHs to have carcinogenic potential and has developed potency equivalency factors (PEFs) for 23 PAHs by comparing the cancer activity of the chemicals relative to benzo[a]pyrene. The USEPA is proposing a RFP approach to assess cancer risks from whole mixtures of PAHs and to expand cPAHs from seven to 24 compounds. Nevertheless, cPAHs typically drive site risks and remediation because of their high cancer potencies and toxicity. This paper describes a human health risk assessment (HHRA) for residential properties where widespread soil PAH contaminations were found. The PAH contaminations were predominately in surface soil and high in areas along roadways, intersections and driveways. A HHRA is performed for residents to characterize potential health risks associated with PAH exposure via incidental ingestion of and dermal contact with soil and inhalation of particulates. In order to estimate a range of potential cancer risks for PAH mixtures, we have utilized RPFs developed by the USEPA in 1993 for the seven cPAHs, PEFs developed by the Cal/EPA for the 24 cPAHs, and the proposed RPFs for the 24 cPAHs by the USEPA. Comparisons of PAH cancer risks using different toxicological approaches have implications for future PAH risk assessment.

**366 PAH Concentrations Associated with Use of Coal-Tar-Based Sealcoat and Relevance for Human Exposure Pathways** B. Mahler, US Geological Survey, Water Resources Discipline; P. Van Metre, US Geological Survey; E.S. Williams, Baylor Univ, Baylor Univ. Coal-tar-based sealants applied to the asphalt pavement of many parking lots, driveways, and playgrounds contain 20 to 35% coal tar (CT) pitch, a known human carcinogen. CT-based sealants typically contain about 50,000-100,000 mg/kg PAH (sum of 16 PAH compounds); asphalt-based sealants, in comparison, contain about 50-100 mg/kg PAH. Pathways by which humans might be exposed to PAHs from CT-based sealants include dermal contact, inhalation, and non-dietary ingestion. This exposure might occur through contact with several media: the pavement surface (dermal), pavement dust (dermal, inhalation, ingestion), soil near sealed pavement (dermal, ingestion), settled/resuspended house dust (dermal, ingestion, inhalation), and air over sealed pavement (inhalation). Median T<sub>12</sub>-PAH (sum of concentrations of 12 PAH compounds) in dried sealant scraped from CT-sealed pavement and in dust swept or vacuumed from the pavement were about 19,000 and 4,000 mg/kg, respectively; T<sub>12</sub>-PAH in scrapings and dust from unsealed pavement were typically less than 50 mg/kg. The median T<sub>12</sub>-PAH in soil near CT-sealed pavement was 80 mg/kg, about 8 times the median in soil near unsealed pavement (< 10 mg/kg). The median T<sub>12</sub>-PAH concentrations in settled house dust from apartments adjacent to CT-sealed parking lots was about 130 mg/kg, compared to a median concentration of < 10 mg/kg from apartments adjacent to unsealed lots. The median concentrations of T<sub>8</sub>-PAH (sum of eight PAH compounds) in the gas phase 1.3 meters above CT-sealed parking lots was 120 ng/m<sup>3</sup>, about four times the median concentration above unsealed parking lots (29 ng/m<sup>3</sup>). Because carcinogenic PAHs are considered non-threshold carcinogens (any additional exposure is associated with increased risk), these values indicate that potential exposure to PAHs by dermal contact, ingestion, and inhalation might be substantially greater in areas where pavement is sealed with CT-based sealants.

**367 Assessing the Two-Year Change in Serum PFC Levels in an Exposed Community in Minnesota** C. Huset, MN Dept of Health, Organic Chemistry Unit; J. Nelson, MN Dept of Health, Chronic Disease and Environmental Epidemiology; P. Swedenborg, MN Dept of Health, Organic Chemistry Unit; J. Johnson, MN Dept of Health, Chronic Disease and Environmental Epidemiology. The Minnesota Dept of Health (MDH)

conducted a human biomonitoring pilot project in 2008/2009 that measured serum levels of seven perfluorochemicals (PFCs) in 196 residents of the East Metro of Saint Paul, Minnesota known to have been exposed to drinking water contaminated with PFCs. Concentrations of PFCs in serum were found to be higher than the national average and strongly associated with the concentration of PFCs in drinking water. Since measures have been in place in these communities since 2005 to reduce exposure to PFCs through contaminated drinking water, it is expected that concentrations of PFCs in serum should be declining. In 2010/2011, MDH followed up with the same residents to determine the two-year change in serum levels of PFCs to assess whether efforts to reduce exposure from drinking water have been successful. The 186 participants from the 2008-2009 study who agreed to future contact were asked to participate; of these, 164 (88%) consented, filled out a questionnaire, and provided a second blood sample. Serum samples were analyzed by the MDH Public Health Laboratory for 7 PFCs by solid phase extraction and liquid chromatography tandem mass spectrometry. The concentrations of perfluorobutanoate, perfluoropentanoate, perfluorohexanoate, perfluorooctanoate, perfluorobutane sulfonate, perfluorohexane sulfonate and perfluorooctane sulfonate in serum samples were determined using matrix matched calibration curves and stable isotope labeled internal standards. The limit of detection for the method was 0.1 ppb. We will present results from this follow-up study and compare to the results from the 2008/2009 project.

**368 Prenatal Exposure to Perfluorinated Compounds in China** T. Zhang, Nankai Univ, China, and Wadsworth Center, USA, Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, Nankai Univ; H. Sun, Y. Lin, MOE Key Laboratory of Pollution Processes and Environmental Criteria, Nankai Univ; K. Kannan, Wadsworth Center, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany. Prenatal exposure to perfluorinated compounds in China Tao Zhang,<sup>1</sup> Hongwen Sun,<sup>1,\*</sup> Yan Lin,<sup>1</sup> Kurunthachalam Kannan,<sup>2</sup> <sup>1</sup>MOE Key Laboratory of Pollution Processes and Environmental Criteria, Nankai Univ, Tianjin 300071, China <sup>2</sup>Wadsworth Center, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany, Albany, NY 12201, USA ABSTRACT Perfluorinated compounds (PFCs) are a group of chemicals widely used for many applications. In this study, PFCs were investigated in 30 paired maternal samples (i.e., maternal blood, maternal urine, cord blood, placenta, and amniotic fluid) from China. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA) were detected in all cord blood samples, and maternal blood and placenta samples. The mean concentrations of PFOS and PFOA in cord blood were 3.09 and 1.95 ng/mL, respectively, accounting for 19% of PFOS, and 58% of PFOA in maternal blood. For fetus, the total intakes of PFOS and PFOA during perinatal period were 7176 and 3475 ng, respectively. Based on 280 days prenatal period, the daily intake for fetus were 25.6 ng PFOS/d and 12.4 ng PFOA/d. The potential for crossing the placental barrier were negatively correlated with carbon chain length of C8-C12 PFCs. The efficiency for placental transfer of PFOA is higher than PFOS, similarly, the efficiency for placental transfer of branch PFOS is higher than linear PFOS. Cross all human urine, PFOS was found at the highest mean concentration with the range of 4.60-15.0 pg/mL, followed by PFOA (1.77-5.24 pg/mL); PFOA was the major PFC in amniotic fluid with the level of 14.8 pg/mL, followed by perfluorobutyric acid (PFBA) (7.02 pg/mL) and PFOS (6.78 pg/mL). Urinary excretion of PFOS and PFOA were 6.90, and 2.90 ng/d for newborn mother, respectively, which account for 7% of daily intake of PFOS, and 12% of PFOA intake. Our results suggested that urine is a potential excretion pathway for adults. Maternal loss of PFOS and PFOA during each childbearing were 15,400 and 5070 ng, respectively. This is the first time to report PFDoDA concentration in humans, and this is the first to assess human exposure to PFCs to Chinese fetus.

**369 Analysis of Dialkyl Phosphate Metabolites of Organophosphate Pesticides in Human Urine** D. Wang, California Dept of Public Health, Environmental Health Laboratory Branch; P. Behniwal, California Dept of Public Health, Environmental Health Laboratory Branch; R. Fan, Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, College of Life Science, South China Normal Univ, California

Dept of Public Health, Environmental Health Laboratory Branch; J. She, California Dept of Public Health, Environmental Health Laboratory Branch. Organophosphate (OP) pesticides continue to be used on crops in agriculture and for pest control in residential settings in the United States. Acute and chronic exposures to OP insecticides have been reported to cause adverse health effects. Most of the OP pesticides used are metabolized to up to six common urinary dialkyl phosphate metabolites (DAPs). Quantification of DAPs provides information on continuous exposure to most OP pesticides. In this study we have developed a novel method for the analysis of human urinary DAPs by using isotope dilution and gas chromatography/tandem mass spectrometer (GC/MS/MS). The sample preparation procedure included lyophilization, derivatization, and clean-up by a dual layer of solid phase extraction (SPE) cartridges, which effectively eliminated matrix interferences and yielded satisfactory results with lower limits of quantification (0.10 ng/mL) for the method. The relative standard deviations were lower than 16% from analysis of three concentrations (3.0, 15 and 30 ng/mL) of DAPs spiked in 2.0 mL urine. The measurement of DAPs in proficiency test samples generated results close to the certified values. The urine matrix might cause variations of analytical results of dimethyl phosphate (DMP), and further studies are being performed to eliminate such matrix effects. The method is being used in several projects in California Environmental Contaminant Biomonitoring Program.

### 370 PCBs and Their Hydroxylated Metabolites in Children and Their Mothers Living in Urban and Rural Communities R. Marek, Univ of Iowa, Civil & Environmental Engineering, The Univ of Iowa, Civil and Environmental Engineering; S.N. Spak, Univ of Iowa, Civil & Environmental Engineering; P.S. Thorne, The Univ of Iowa, Occupational and Environmental Health; J. DeWall, The Univ of Iowa, Occupation and Environmental Health; K.C. Hornbuckle, Univ of Iowa, Dept of Civil & Environmental Engineering. We have analyzed human blood serum collected since 2008 from adolescent children and their mothers in urban East Chicago, Indiana (n >100) and rural Columbus Junction, Iowa (n >100) for all 209 PCB congeners and 11 hydroxylated PCBs (OH-PCBs). East Chicago is a heavily-industrialized residential community on the southwestern shore of Lake Michigan. Bisecting the area, and passing within 2 km of two schools, is the Indiana Harbor and Ship Canal from which about 7 kg PCBs per year volatilize. In contrast, residents of the rural Columbus Junction area have no known PCB exposure from current or past industrial sources. We used highly selective methods (EPA Method 1668 modified for GC/MS/MS) and a full suite of quality control measures (SRM, surrogate recoveries, laboratory blanks, internal standard method) to optimize the analytical method for our target compounds. Data of each sample population approximates a Poisson distribution with a long tail. Analysis of the first year of samples suggests that East Chicago residents have a higher baseline than Columbus Junction residents although the two communities exhibit similar median PCB concentrations. Residents of both communities had similar levels of OH-PCBs serum concentrations. Interestingly, though mothers almost always had approximately double the PCB body burden of their children, some children had equal to or double the OH-PCB body burden of their mothers. Differences in PCB and OH-PCB body burdens were discovered between male and female children. Following analysis of a second round of blood sampling one year later, these trends are examined for continuity within the second year of data. We present the results of this analysis and possible implications of our findings.

371 **Assessment of Human Exposure to Chlorinated and Brominated Compounds in Ghana** K.A. Asante, Ehime Univ, Center for Marine Environmental Studies (CMES), Ehime Univ, Center for Marine Environmental Studies; S. Takahashi, Ehime Univ, Center for Marine Environmental Studies (CMES); T. Isobe, Ehime Univ, Senior Research Fellow Center; T. Imai, G. Devanathan, Ehime Univ, Center for Marine Environmental Studies (CMES); O.D. AnsaAsare, CSIR, Water Research Institute; S. AduKumi, Environmental Protection Agency, Chemicals Management; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies (CMES). Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are widely used in polymers and consumer products to slow down the combustion of these materials. Among BFRs, PBDEs have received the most attention because of their environmental ubiquity, persistence and also possess a notable potential of bioaccumulation. As a result, the Penta-BDE and Octa-BDE commercial mixtures have been banned by the European Union and voluntarily

withdrawn from the marketplace in the United States. Polychlorinated biphenyls (PCBs) on the other hand were historically used in various applications such as hydraulic fluid additives, insulating oil for transformers, capacitors, plasticizers, etc. The widespread occurrence of flame retardants in the indoor and outdoor environment requires constant monitoring as human exposure to these chemicals is of particular concern. These contaminants were measured in human breast milk and tilapia fish samples collected from Ghana. Mean concentrations of PCBs (62 ng/g lw), PBDEs (4.5 ng/g lw) and HBCDs (0.54 ng/g lw) measured in breast milk samples using GC-MS and LC-MS/MS, were unexpectedly high, albeit Ghana is a non-industrialized country when compared with many of the European and Asian countries. The health risk of infants and adults were calculated through mothers' milk and fish consumption, respectively using hazard quotients (HQs). Estimated HQs showed that all the mothers had HQ values exceeding the threshold of 1 for PCBs, indicating potential health risk for their newborns. However, the HQs through fish were all below 1, suggesting less potential health risk of these contaminants to Ghanaians via fish consumption. Our fish findings indicate a moderate contamination level; however, continuous study of the temporal trends of PCBs and BFRs in Ghana is needed to evaluate long-term impacts to human health via fish consumption since fish is seen as the most important source of animal protein in Ghana. Ghana does not produce PCBs, however, there is illegal or uncontrolled usage of PCB oils and thus PCBs in dirty oils and obsolete equipment should be of concern as potential sources in Ghana. Furthermore, e-waste recycling with little or no experience in safe handling could be a threat to Ghana noted for unregulated disposal and incessant burning of e-waste. This is the first study to report BFRs in human breast milk and fish from Ghana and arguably from Africa.

372 **Phthalate Metabolites in Human Urine from China and Potential Sources of Phthalate Exposure** Y. Guo, Q. Wu, K. Kannan, Wadsworth Center, New York State Dept of Health. Urinary phthalate metabolites can be used as biomarkers to reflect recent human exposure to phthalates. In this study, 14 phthalate metabolites were analyzed in 183 urine samples collected in 2010 from Shanghai, Guangzhou, and Qiqihaer, China. To evaluate potential human exposure sources, 75 indoor dust samples collected from six cities in China were also collected and 9 phthalates were determined. Phthalate metabolites were detected in all urine samples, with a total concentration ranging from 18.6 to 3160 ng/mL (median: 331 ng/mL). Mono-n-butyl phthalate (mBP) and mono-2-isobutyl phthalate (miBP) were the major metabolites found in urine. In dust samples, bis(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP) and di-iso-butyl phthalate (DIBP) were the predominant compounds found; concentrations of DEHP (median: 228 µg/g) were 10 times higher than the concentrations of DBP (20.1 µg/g) and DIBP (17.2 µg/g), and the concentrations of these three compounds were 1000 times higher than the concentrations of the other 6 phthalates analyzed. Based on the concentrations of phthalate metabolites in urine and of phthalates in house dust, our results indicated that indoor dust accounted for less than 0.2% of the total intakes reported for dimethyl phthalate (DMP) and DEP, less than 2.2% of the intakes for DIBP and DBP, and 2%-5% of the intakes for DEHP in China. Furthermore, dietary intake was the main source of exposure to all phthalate esters, especially for DEHP (> 95%).

373 **One-box Population-level Pharmacokinetic Models for Interpretation of Biomonitoring Data for Persistent Pollutants** M. MacLeod, Stockholm Univ, Dept of applied environmental science; R. Ritter, M. Scheringer, ETH Zurich; C. Moeckel, K.C. Jones, Lancaster Univ; K. Hunebruhler, ETH Zurich. Analysis of persistent organic pollutants (POPs) in human blood and milk is a key component of the global monitoring plan that will assess the effectiveness of the Stockholm Convention. At the same time, there are an increasing number of biomonitoring programs underway at the national level in many jurisdictions. There is a need to develop quantitative modeling tools to extract as much meaningful information as possible from the levels and trends found in these biomonitoring data. Here, we present two novel applications of a one-box pharmacokinetic model applied at the population-level to interpret biomonitoring data. In the first case, we examine the significance of time-trends of POPs in human milk in a population that has lived entirely in a post-ban situation where exposures are falling exponentially. We use biomonitoring data for DDT and DDE from Sweden that represent this situation. Our modeling demonstrates that in this special case the time trend in the biomonitoring data directly corresponds to the



time trend for reduction of exposure to DDT and DDE. In the second case study we analyze biomonitoring data from a population with some members who have lived through increasing, peak and decreasing regimes of exposure. In this case study we use two sets of biomonitoring data for polychlorinated biphenyls (PCBs) from a broad cross-section of the UK population. We demonstrate that a one-box population-level pharmacokinetic model can be fit to such biomonitoring data in two dimensions simultaneously to describe both the time course of average bodyburden in the population, and the age – concentration relationship within each set of cross-sectional data. Applied in this way, the model reconstructs the time course of exposure of the population, and estimates the half-time for intrinsic elimination of POPs from the bodies of the members of the population at background exposure levels. Results from our case study for PCBs in the UK population indicate that the maximum intrinsic half-time for elimination of even the most persistent and hydrophobic PCBs is about 15 years. We hypothesize that this half-time corresponds to elimination by non-metabolic processes, and that it represents a baseline human elimination rate for POPs.

**374 Organohalogenated Contaminants and Their Metabolites in Human Serum from Obese Patients** A.C. Dirtu, Toxicological Centre, Univ of Antwerp, Dept of Pharmaceutical Sciences; E. Dirinck, Antwerp Univ Hospital and Univ of Antwerp, Dept of Endocrinology, Diabetology and Clinical Pharmacology; H. Neels, Toxicological Centre, Univ of Antwerp, Dept of Pharmaceutical Sciences; L. Van Gaal, P. Jorens, Antwerp Univ Hospital and Univ of Antwerp, Dept of Endocrinology, Diabetology and Clinical Pharmacology; A. Covaci, Toxicological Centre, Univ of Antwerp, Dept of Pharmaceutical Sciences. Due to their persistent, bioaccumulative and toxic properties, persistent organic pollutants remain important to be evaluated in humans. In the present study, we evaluated the levels and profiles of several PCB congeners and their hydroxylated metabolites (HO-PCBs), polybrominated diphenyl ethers (PBDEs), pentachlorophenol (PCP) and some organochlorine pesticides (OCPs) in obese humans with an increased body mass index. We collected serum from obese individuals visiting the Obesity and Diabetes Clinic of the Univ Hospital of Antwerp, Belgium. These patients participate in a weight loss program by undergoing either bariatric surgery or through diet and physical exercises. Serum was sampled before the patients entered the program (N=95) and after approximately 6 (N=55) and 10 months (N=39), respectively. Time dependencies were tested for the most important PCB congeners (CB 118, 153, 180 and 170) and indicated that PCB levels increased significantly for each patient, independent of the treatment method employed, even when the results were adjusted for the lipid content. These results may be important, since organochlorines released in plasma during weight loss have been associated with the decrease in the serum thyroxine concentrations. Additionally, HO-PCBs were used to evaluate the influence of the treatment method on the metabolic capacity of the patients. Even if less significant, an increasing level with time was observed only for some HO-PCB metabolites (3HO-CB153, 4HO-CB107 and 4HO-CB146). Moreover, a change in time of their profile was noticed, mainly through an increasing contribution of 3'HO-CB138 to the total HO-PCBs concentration. The most important OCP was *pp'*-DDE, a metabolite of *pp'*-DDT, while hexachlorobenzene, hexachlorocyclohexanes and chlordanes were measured at lower levels. The levels of PBDEs were in most of the cases close to the method limit of quantification and were thus less important compared to other classes of neutral contaminants. Apart from HO-PCBs, the most important phenolic contaminant was PCP with median concentrations of 450 pg/mL for patients before entering the program and nearly similar median concentrations of 520 pg/mL at 6 and 10 months after the program was initiated. Our results corroborate the hypothesis that some endocrine disrupting chemicals not only might play a role in the occurrence of obesity, but also in the metabolic derangements seen during fat loss.

**375 Adverse Outcome Pathways During Early Fish Development: A Conceptual Framework for Identification of Chemical Screening and Prioritization Strategies** D. Volz, Univ of South Carolina, Univ of South Carolina, Dept of Environmental Health Sciences; S. Belanger, The Procter & Gamble Company; M. Embry, ILSI Health and Environmental Sciences Institute; S. Padilla, US Environmental Protection Agency; H. Sanderson, Aarhus Univ; K. Schirmer, Swiss Federal Institute of Aquatic Science and Technology; S. Scholz, UFZ – Helmholtz Center for Environmental Research; D. Villeneuve, US Environmental Protection Agency. The fish early life-stage (FELS) test guideline (OECD 210 or OCSP 850.1400) is

the most frequently used bioassay for predicting chronic fish toxicity and supporting aquatic ecological risk assessments around the world. For each chemical, the FELS test requires a minimum of 360 fish and one to three months from test initiation to termination. While valuable for predicting fish full life-cycle toxicity, FELS tests are labor- and resource-intensive and, due to an emphasis on apical endpoints, provide little to no information about chemical mode-of-action. Therefore, the development and implementation of alternative testing strategies for screening and prioritizing chemicals has the potential to reduce the cost and number of animals required for estimating fish early life-stage toxicity and, at the same time, provide insights into mechanisms of toxicity. Using three reference chemicals with well-established yet distinct adverse outcome pathways (AOPs) in early life-stages of fish, we proposed FELS-specific AOPs as conceptual frameworks for identifying useful chemical screening and prioritization strategies. The reference chemicals selected as case studies were a cardiotoxic aryl hydrocarbon receptor agonist (2,3,7,8-tetrachlorodibenzo-*p*-dioxin); neurotoxic acetylcholinesterase inhibitor (chlorpyrifos); and narcotic surfactant (linear alkylbenzene sulfonate). Using qualitative descriptions for each chemical during early fish development, we developed generalized AOPs and, based on these examples, proposed a three-tiered testing strategy for screening and prioritizing chemicals for FELS testing. Linked with biologically based, concentration-response models, a tiered testing strategy may help reduce the reliance on long-term and costly FELS tests required for assessing the hazard of thousands of chemicals currently in commerce.

**376 Differential Regulation of Resource Genes in Carbon Nanotube Exposed *Daphnia magna*** A. Edgington, Clemson Univ, Biological Science and CU-ENTOX, Clemson Institute of Environmental Toxicology; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX). Engineered carbon nanotubes (CNTs) have been shown to become stable in aqueous suspensions once functionalized with natural organic matter (NOM). This allows the material to become bioavailable to many aquatic organisms, including the filter feeding zooplankton *Daphnia magna*. Previous research has shown microscopically that once ingested the CNTs can impact the gut tract of *D. magna* and this may interfere with the normal digestive processes. One area of digestion that may be interrupted is the peritrophic matrix (PM) layer within the gut. The PM is a layer of chitin and proteins produced by the lumen. Its function is not completely known, but it has been shown to aid in digestion and protect the organism from toxic chemicals and pathogens. Furthermore, researchers have also shown that there are as many as 14 genes differentially regulated when *D. magna* is presented with different qualities of food. The objectives of this research were to determine the differential regulation of peritrophic matrix and resource genes to carbon nanotube exposed *D. magna*. Seventy-two hr old *D. magna* were exposed to 0, 0.5, and 2.0 mg/L multi-walled (MWNT) and single-walled nanotubes (SWNT) for 24 hrs. Organisms within treatments were pooled and processed for Real Time PCR. The results show that both SWNT and MWNT exposure has an effect on PM and resource gene expression. Genes associated with PM production were upregulated with MWNT and SWNT exposure. Resource genes were differentially expressed with SWNT and MWNT exposure with an increase in genes associated with metabolism (ATP synthase) and molting (cuticle protein). The results from this study suggested that the physical stress that MWNTs and SWNTs have on *D. magna* gut induces a biochemical response. The gene differentiation suggest that *D. magna* respond to the physical stressor by increasing the protective PM layer within the gut tract and changing expression of resource genes to handle the nutrient poor MWNTs and SWNTs.

**377 Development of a Knowledge-base Web Application for Adverse Outcome Pathways: The AOP App** N. Garcia-Revero, Jackson State Univ; P. Gong, SpecPro Inc., Environmental Services; K.A. Gust, US Army, Engineer Research and Development Center, Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, ERDC-EL-EP-P; T. Habib, BTS; D.R. Johnson, US Army Engineer Research and Development Center, Environmental Laboratory; M. Mayo, US Army ERDC; B.C. Suedel, US Army Engineer Research and Development Center, CEERD-EP-R, US Army Engineer Research and Development Center, Environmental Laboratory, Waterways Experiment Station EP-R; V. Dickerson, US Army, Engineer Research & Development Center; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division.

Advances in biological sciences and related technologies are changing the types and amount of data that toxicologists can collect and analyze in an efficient, cost-effective, and reliable manner. More recently, “omics” and systems biology approaches have emerged as tools for both basic biology and toxicology/ecotoxicology research. Overall, ecotoxicology in the 21<sup>st</sup> century is characterized by an increased mechanistic understanding of basic biology, and increased ability to analyze complex data, integrate and manage diverse information, and conduct in silico simulations. Furthermore, advances in science and technology are increasing the feasibility to use alternative data/endpoints (e.g., biomarkers, in vitro bioassays, genomics) as a scientifically credible basis for ecological risk assessment (ERA). Recently, a strategy organized around the identification of adverse outcome pathways (AOPs) has gained interest within the ecotoxicology community. It is envisioned that AOP-based QSARs (quantitative structure-activity relationship), in vitro bioassays, and biomarkers, coupled with quantitative extrapolation tools, will assume a more prominent role in ERA as the science of predictive ecotoxicology develops. Our aim is to develop a web application to integrate all the available information and facilitate the use of AOPs. We are coupling “omics” technologies with other physiological measurements and literature research to define and predict AOPs relevant to ERA. We have begun by focusing on the AOPs related to reproduction, liver toxicity, and neurotoxicity. The idea is to characterize and understand the different AOPs of interest, and then upload this information into a knowledge base web application that is under development, the *AOP App*. The *AOP App* will allow users to explore and understand AOPs of interest and allow for users to input data in order to predict the potential toxicity and relation to AOPs of the chemicals of interest. The type of data that the *AOP App* will support will be analogous to the data used to create the database, such as omics data, and physiological measures among other conditions. In summary, the AOP App will provide integrative ecotoxicological utilities for scientists and regulators alike.

**378 Development of a Cell-free Neurochemical Screening Battery to Predict Adverse Outcomes in Mammals, Fish and Birds** N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health; D. Nam, J. Head, Univ of Michigan; C. Murphy, Michigan State Univ. Cell free, in vitro bioassays have been developed in biomedicine to screen, for example, neurotoxic agents and novel pharmaceuticals. These cell-free methods are now being used by the USEPA's ToxCast program, and adaptation/development of such bioassays for predicting adverse outcomes is particularly attractive in ecological risk assessment owing to the lack of high-throughput in vitro screening methods that span several taxa, and the limited ability to conduct whole-animal bioassays. Here we describe a series of in vitro assays that may be used to assess whether contaminants interact with, and possibly disrupt the function of, various neurotransmitters receptors and enzymes that mediate fish reproduction. Specifically, in this presentation it was hypothesized that metals ( $Hg^{2+}$ ,  $CH_3Hg^+$ ,  $Pb^{2+}$ , total Se,  $Sn^{2+}$ ,  $As^{3+}$ ,  $Cd^{2+}$ ,  $Cr^{6+}$ ,  $Mn^{2+}$ ) will emerge to inhibit binding to the muscarinic acetylcholine receptor (mAChR) and N-methyl-D-aspartate receptor (NMDAR) in brain cortical tissues collected from several fish (goldfish, perch, lemon shark, mako shark), birds (chicken, bald eagle), and mammals (mouse, polar bear, mink, common dolphin, Atlantic white-sided dolphin). First, saturation binding curves were developed from each species to calculate mean receptor density ( $B_{max}$ ) and ligand affinity ( $K_d$ ). Next, samples were exposed to the aforementioned metals at several concentrations to derive IC<sub>50</sub> (inhibition concentration 50%) values. Based on IC<sub>50</sub> values and resulting inhibition constants ( $K_i$ ), generalized rank-order potencies for metals and species were developed and will be presented. These comparative results concerning interspecies and inter-metals differences potentially provide a high throughput, in vitro framework for evaluating neuroendocrine risk to ecological organisms. Further, we have developed (and continue to develop) cell-free in vitro methods for several other neuroendocrine receptors and enzymes (e.g., estrogen receptor, androgen receptor, GABA receptor) of concern to vertebrate reproduction, with the ultimate goal of modeling the in vitro results to individuals and populations.

**379 What is a Model Species? Linking Sublethal Stress Responses to Population Impacts via Computational Modeling in Great Lakes Fish** C.A. Murphy, Michigan State Univ, Dept of Fisheries and Wildlife, Lyman Briggs College, Michigan State Univ, Lyman Briggs College; S. Smith, Michigan State Univ; J. Head, Cooperative Institute for Limnology and Ecosystems Research; N. Basu, Univ of Michigan; R. Goetz, M. Carvan, Great Lakes WATER Institute; S. Sitar, Michigan Dept of Natural

Resources. The development of appropriate extrapolation models to link key molecular initiating events to adverse outcomes is a crucial component of the adverse outcome pathway (AOP) framework. Only recently, a few of these models, that convert sublethal responses of stressors to endpoints that can be interpreted at higher levels of biological organization, have been developed – and only in a few model species, eg fathead minnow. Very few extrapolation models exist for larger, long-lived, commercially valuable species. Here we present two “in progress” case studies on species of relevance to the Great Lakes, lake trout (*Salvelinus namaycush*) and yellow perch (*Perca flavescens*). We compare and contrast the sublethal effects of two different stressors (sea lamprey parasitism and mercury contamination) on the Hypothalamus-Pituitary-Gonadal (HPG) axis of lake trout and perch and illustrate different modeling approaches to convert the effects of these stressors that often manifest at the suborganism level, to population relevant points such as fecundity and growth. Such endpoints can then be input into population models to project population effects. In these models, we attempt to incorporate differences that manifest as a result of differences in life history within and between species. We discuss the difficulties and assumptions that are necessary to make between population and between species comparisons. With further refinement, our approach may be useful to determine the harmful effects of stressors across a wider array of populations and species.

**380 Incorporation of Predictive Population Modeling into the AOP Framework: A Case Study with White Suckers Exposed to Pulp Effluent** D.H. Miller, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; J.E. Tietge, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; M.E. McMaster, Environment Canada, National Water Research Institute; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology; X. Xia, Computer Sciences Corporation; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division. A need in ecological risk assessment is the ability to create linkages between chemically-induced alterations at molecular and biochemical levels of organization with adverse outcomes in whole organisms and populations. A predictive model was developed to translate changes in the fecundity of a breeding population of white sucker (*Catostomus commersoni*) collected in the field to alterations in population growth rate. Individual-level responses of fish exposed to pulp mill effluent at a study site in Jackfish Bay, Lake Superior, were used to demonstrate the model's capability to project alterations in population status. The Jackfish Bay study site also has monitoring data for biochemical endpoints of interest in the white sucker, including steroid measurements amenable to interpretation through different adverse outcome pathways (AOPs) for reproductive effects. Linkage of the population model to biochemical measurements relevant to adverse outcomes can facilitate extrapolation of data from the Jackfish Bay study site to other white sucker populations at sites that are less data rich. Application requires only a life table for the organism of interest, a measure of carrying capacity for the given population, and estimation of the effect of stressors on vital rates within the fish of the study population. This abstract does not necessarily reflect USEPA Policy.

**381 Methylmercury Effects of Larval Fish in the Lab: Modeling to the Population Level** M.J. Carvan, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences, Univ of Wisconsin-Milwaukee, Childrens Environmental Health Sciences Center; C.A. Murphy, Michigan State Univ, Dept of Fisheries and Wildlife, Lyman Briggs College, Michigan State Univ, Lyman Briggs College; D. Weber, Univ of Wisconsin-Milwaukee, Childrens Environmental Health Sciences Center, Univ of Wisconsin-Milwaukee, Great Lakes Water Institute, Univ of Wisconsin-Milwaukee; F. Goetz, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences; J. Head, Univ of Michigan, Cooperative Institute of Limnology and Ecosystem Research, Univ of Michigan, Cooperative Institute for Limnology and Ecosystem Research; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. Zebrafish have been utilized as a model for developmental exposures to mercury and are a useful model for both human and other vertebrate species due to short intergenerational periods. Using startle response and learning behaviors, we examined the effects of developmental waterborne and maternal-derived exposures to

mercury on both short- and long-term outcomes. These behavioral changes were related to specific anatomical, physiological, and molecular initiating events and we have investigated the ability of specific factors to mitigate these behavioral deficits. Mercury exposures affect 3 parameters: latency of reaction, maximum escape velocity, and duration of escape swim. In mercury-exposed fish, reaction time to a vibrational stimulus is slower, maximum escape velocity is slower, and duration of escape swim is longer than control fish. Reaction to a visual stimulus by adult fish developmentally exposed to mercury is also decreased. Learning in a spatial alternation task is impaired in fish developmentally exposed to mercury. Recent data suggests that selenium compounds in the diet may be important in mitigating specific neurobehavioral deficits due to MeHg exposure. Co-exposures of methylmercury (MeHg) and selenomethionine (SeMet) during early embryogenesis did result in an improvement in visual startle responses, including improvements in K<sup>+</sup> efflux in retinal bipolar cells. In contrast, similar co-exposures of MeHg and SeMet did not result in improvements in learning or cell body numbers in the telencephalon. These subtle changes in behavior and learning were then incorporated into an individual-based model to predict the effects on survival and growth of a cohort – endpoints that can be modeled to determine population relevant effects. Future studies will perform the same suite of experiments on larval perch to determine the efficacy of extrapolating across species and to link adverse effects from molecular initiating events to population level impacts in a Great lakes species of ecological and economic importance, via an adverse outcome pathway framework.

**382 Estrogenic Effects on Largemouth Bass at Multiple Biological Levels from Chronic Ethinyl Estradiol Exposure Following an Adverse Outcomes Paradigm** D.M. Papoulias, US Geological Survey, Columbia Environmental Research Center; C.A. Richter, USGS – Biological Resources Division, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; M. Wildhaber, US Geological Survey, USGS; D.E. Tillitt, US Geological Survey, Columbia Environmental Research Center; N.D. Denslow, A.C. Mehinto, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology; K.J. Kroll, Univ of Florida, Physiological Sciences. Feminized male fish from marine and freshwaters have been reported with increasing frequency. Exposure to estrogenic chemicals is suspected based on evaluation of known biomarkers of estrogenicity such as male vitellogenin production and appearance of ovo-testes, among others. Although useful for evaluating individual exposure, it is generally not known how well such diagnostic measures predict adverse effects on functional outcomes such as reproduction. We studied the effects of an environmentally-relevant concentration of EE2 on virgin adult largemouth bass in outdoor mesocosms. The reproductive success of exposed and unexposed bass was compared at the end of the exposure. We evaluated effects of exposure at multiple levels of biological organization along the AOP from the molecular level through the organismal level. Fecundity and fertility data were then used in a simple model to predict population size into the future. Mean egg production was 30% lower in ponds when fish had been exposed to EE2 for 18 months, but not significantly different from controls. Plasma vitellogenin was greatly increased in both sexes and gonads of EE2-exposed fish were underdeveloped at the organ level. Unexpectedly, testicular oocytes were not observed in any testes examined despite complete sectioning and evaluation of an entire lobe from each of 90 individual males. The molecular results were in part consistent with our findings at higher levels but also provided new insights and identified potentially new biomarkers. Our results are timely because feminized largemouth bass have been reported for several drainages in North America but as yet, definitive causes have not been identified. Ethinyl estradiol at an environmental concentration similar to or higher than that used in this study is commonly found in these same drainages but effects of chronic exposure on fish reproduction are not fully understood. This study provides a good example of the use of the AOP approach as it ties together multiple cause and effect events that together lead to impaired reproduction from xeno-estrogen exposure.

**383 Contamination from Formerly Used Defense Sites in Alaska Native Villages Along Norton Sound and St. Lawrence Island** E.A. von Hippel, Univ of Alaska Anchorage, Dept of Biological Sciences; P.K. Miller, C. Keane, Alaska Community Action on Toxics; R. Bernhardt, Univ of Alaska Anchorage; V. Waghiyi, Alaska Community Action on Toxics; L. Buck, Univ of Alaska Anchorage. Alaska has approximately 700 formerly used defense sites (FUDS), many of which are located in close proximity to Alaska Native

communities in remote parts of the state. Some communities have raised concerns that legacy contaminants from FUDS may be contaminating their water supplies and traditional foods. Because Alaska Natives rely heavily on subsistence harvest of top carnivores, they have the potential to ingest unsafe quantities of contaminants that have bioaccumulated and biomagnified in the lipid-rich Arctic food web. Here we report on results examining contaminant load and endocrine disruption in freshwater fishes from aquatic sites near FUDS in Alaska Native villages along Norton Sound. At the request of local communities, the threespine stickleback was used to test for estrogenic and anti-estrogenic contaminants using the vitellogenin biomarker, and to test for androgenic and anti-androgenic contaminants using the spiggin biomarker. Many male stickleback from the sites in the villages of Wales and Unalakleet expressed elevated levels of vitellogenin, demonstrating that they had been exposed to estrogenic xenobiotics. The US Army Corps of Engineers and the Alaska Dept of Environmental Conservation cleaned the White Alice Communication site at Northeast Cape on St. Lawrence Island in 2003. Despite this cleanup, we found that Alaska blackfish downstream of the site contained 7-35 ppb total PCBs in their tissues. PCB Aroclor 1260 was the most common parent product, indicating that the majority of the contamination originates in the remediated site and is not acquired through global transport. These results suggest that FUDS pose a risk to the health of local inhabitants, and that detailed characterization of these sites is important to prioritize cleanup efforts.

**384 Overview of the Aquatic Ecotoxicology of the Explosives TNT, RDX and HMX** G. Lotufo, US Army Engineer Research and Development Center, Environmental Laboratory, U S Army ERDC; G. Rosen, SPAWAR Systems Center Pacific, Environmental, SPAWAR Systems Center Pacific, Scientist; J.B. Belden, Oklahoma State Univ, Dept of Zoology; B. Wild, SPAWAR Systems Center Pacific. To support the assessment of risk associated with the presence of explosives in aquatic environments, laboratory-based toxicity data have been derived for a variety of freshwater and marine fish and invertebrate species and endpoints in exposure to spiked water. Most of those studies have involved the use of unrealistically high water or sediment concentrations in order to derive toxicity benchmarks. TNT and its transformation products caused decreased survival at concentrations ranging from < 1 to 10 mg/L. Species vary in their relative sensitivity to TNT and its major aminated transformation products. RDX caused decreased survival of some fish and invertebrate species at similar concentrations than those reported as acutely toxic for TNT, while some fish species and invertebrates exhibited decreased survival in exposure to concentrations approaching its solubility limit. HMX caused no decreased survival in saturated solution. Because of high biotransformation rates, body residues of most nitroaromatic compounds in exposed animals are reported at levels substantially lower than those predicted by their hydrophobicity. Bioconcentration factors were < 1 ml/g for HMX and approximately 2 ml/g for RDX. Higher values, ranging from 1 to 13 ml/g, were obtained for TNT and its transformation products. Therefore, explosives in tissues of aquatic receptors are expected to exceed the concentration in the surrounding water by only less than one order of magnitude. Uptake of TNT in fish and aquatic invertebrates resulted in substantial bioaccumulation of nonextractable compounds which have lower rates of elimination than the parent compound. For fish, aqueous exposure is likely the dominant route of exposure for explosive compounds, with dietary uptake providing only minimal contribution. Elimination half-lives ranged from a fraction of one hour to a few hours, requiring constant exposure to contaminated water for the retention of explosives in the tissues. Explosives have been detected in surface water from contaminated field sites. However, a lack of adequate information on spatial distribution of contamination and potential to elicit toxicity at contaminated sites preclude an accurate evaluation of the local and global environmental significance of the presence of explosives in aquatic systems. A laboratory experiment simulating real-world exposures concluded that the presence of UXOs in marine environments is unlikely to result in sufficient exposure to cause biological effects to aquatic invertebrates and fish. Verification of this conclusion, however, should be pursued by determining exposure risk at field sites where underwater explosives are present.

**385 Multiple Environmental Stressors in the Western Fence Lizard (*Sceloporus occidentalis*)** C. McFarland, US Army Public Health Command (provisional), and Preventive Medicine, US Army Public Health Command, Health Effects Research Program; L. Talent, Oklahoma State Univ; K. Gust, US Army Corps of Engineers; M. Quinn, US Army Public



Health Command; M. Bazar, US Army PHC; M. Wilbanks, E. Perkins, US Army Corps of Engineers; R. Gogal, Univ of Georgia; M. Johnson, US Army PHC. Wildlife experience a variety of stressors, with exposure to potentially toxic chemicals being only one of them. The relative influence of chemical exposure with other stressors such as food limitation and pathogenic infection is a question of management concern, particularly for threatened and endangered species. Contamination of the soil with the explosive 2,4,6-trinitrotoluene (TNT) has been found at military munitions ranges and industrial waste sites where valued reptile species occur. TNT has been shown to cause anemia at high repetitive exposures. In addition, malarial parasites (*Plasmodium* spp.) are common in most lizard families and can also cause anemia. Diet restriction can exacerbate this condition. The present study investigated the combined and separate influence of food limitation, TNT exposure, and *Plasmodium mexicanum* infection in the Western Fence Lizard (*Sceloporus occidentalis*). Three experimental treatment groups were established based on 1) Experiment I – TNT x Food Limitation, 2) Experiment II – Food Limitation x Malaria Infection, and 3) Experiment III – TNT x Malaria Infection. Preliminary results of the pairwise stressors 1) TNT x Food Limitation, 2) Food Limitation x Malaria Infection, and 3) TNT x Malaria Infection are currently completed examining survival, body weight, and cricket consumption. Experiments with TNT exposure demonstrated changes in most of the measured endpoints: approximately 67% in Experiment I and 43% in Experiment III were significantly different. Malaria infection affected cricket consumption, spleen and testes weight, and total WBC counts in Experiments II and III. Limited cricket intake was associated with a loss in body weight, testes and inguinal fat organ weights, albumin and cholesterol blood levels, and total WBC counts in Experiments I and II. Additional results from these experiments will be presented.

### 386 Effects of Dinitrotoluenes on Carbon Mineralization and Microbial Biomass in Sassafras Sandy Loam Soil

M.L. Minyard, Defense Threat Reduction Agency; R.G. Kuperman, US Army Edgewood Chemical Biological Center, Environmental Toxicology, US Army Edgewood Chemical Biological Center, Edgewood Chemical Biological Center, Environmental Toxicology, Edgewood Chemical Biological Center, Environmental Toxicology Branch; R.T. Checkai, The Ohio State Univ, School of Environment and Natural Resources, US Army Edgewood Chemical Biological Center, Environmental Toxicology, US Army Edgewood Chemical Biological Ctr, Dept of Environmental Toxicology; S. Rocheleau, Biotechnology Research Institute, Applied Ecotoxicology; L. Paquet, J. Hawari, Biotechnology Research Institute; G.I. Sunahara, NRC-Biotechnology Research Institute, NRC-Biotechnology Research Institute. We assessed the effects of energetic materials (EM) 2,4-dinitrotoluene (2,4-DNT), 2-amino-4,6-dinitrotoluene (2-ADNT), and 4-amino-2,6-dinitrotoluene (4-ADNT) on basal and substrate-induced respiration (BR and SIR) in Sassafras sandy loam (SSL) soil. Studies were designed to determine toxicity benchmarks for developing Biological Activity-based Soil Screening Concentrations (BA-SSC) and for assessing ecological risks at EM-contaminated sites. EM treatments consisted of freshly-collected SSL soil, amended and thoroughly mixed to prepare nominal concentrations ranging from 10 to 10000 mg EM/kg dry soil, plus carrier (acetone) Control. Preliminary results showed that BR rate was inhibited by 2,4-DNT with the EC20 and EC50 values (mg/kg) of 651 and 2023, respectively on day 7 of soil incubation; and 1033 and 3200, respectively for the 95-h cumulative BR. The initial SIR and biomass C, calculated after 3 h of SIR, were more sensitive endpoints for assessing the effects of 2,4-DNT on carbon mineralization when compared to BR. The respective EC20 and EC50 values (mg/kg) were 20 and 63 for SIR, and 23 and 71 for biomass C. 2-ADNT did not affect BR or SIR (after 10 h) up to and including 10000 mg/kg, but significantly ( $p=0.003$ ) inhibited biomass C at 10000 mg/kg (Lowest Observed Effect Concentration; LOEC). 4-ADNT significantly ( $p=0.009$ ) inhibited BR rate at 10000 mg/kg (LOEC) on day 7, but did not affect the cumulative BR up to and including 10000 mg/kg. These preliminary results are consistent with our earlier studies that assessed the effects of 2,4-DNT, 2-ADNT, and 4-ADNT on decomposition of Orchard grass (*Dactylis glomerata*) straw in similar SSL soil. Both studies suggest that biologically-mediated processes in soil can be inhibited when soil is contaminated with dinitrotoluenes. Our studies also demonstrated that the toxicity benchmarks for some nitroaromatic EM, and corresponding BA-SSC values, can be reliably established for soil biological processes and comply with the stipulations and requirements developed by the US Environmental Protection Agency for terrestrial plant-based or soil invertebrate-based Ecological Soils Screening Levels (<http://cfpub.epa.gov/ecotox/>). Assessment of the

soil microbial activity endpoints can provide valuable information on the effects of EM on critical ecosystem-level processes, such as energy cycling, complementing and expanding upon ecotoxicological significance of data from standardized single-species toxicity tests.

**387 Effect of Kinetics on the Resistance to Desorption of Munitions Constituents (MC) from Soil** R. Gonzalez, K. Michelson, Univ of Delaware, Dept of Civil and Environmental Engineering; D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering; H.E. Allen, Univ of Delaware, Civil & Environmental Engineering Dept. The majority of research has focused on the adsorption of munitions constituents (MC) onto soils, but not on the more environmentally relevant desorption and resistance to desorption, which ultimately determines the environmental threat of these contaminants and their transport through the groundwater. The kinetics of desorption of munitions constituents study has applicability in formulating accurate risk assessments to different operational ranges. We conducted batch experiments near 1:1 soil to solution ratios reflecting field conditions that contained HMX, RDX, nitroglycerine (NG), TNT and 2,4-dinitro toluene as MC. The soils used varied from 0.1 to 12.5 percent organic matter and the experiment involved one adsorption step followed by four consecutive desorptions. Adsorption experiments were conducted on samples that were mixed for 2, 5, 10, and 30 days, and the changes in the adsorption isotherm for the low organic matter content soil was small for the MC with the exception of NG, while larger changes were observed for all MC in the high organic matter content soil. Degradation was the principle cause of these changes and transformation products were observed after each desorption step. For each adsorption experiment desorption steps were carried out for 1, 12, 24, and 72 hours. The most important observation was that the isotherms for each desorption time had the same slope. This implies that desorption hysteresis is not changed by the length of the desorption time, regardless of the initial adsorption time.

**388 A Site Transformation Model of Adsorption-Desorption Hysteresis** D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering. The non-reversibility of adsorption and desorption, i.e., the fact that desorption isotherms do not conform to the adsorption isotherm is commonly observed. This hysteresis has been variously attributed to kinetic effects, i.e., the hysteresis would disappear if the desorption time were long enough, or to the existence of a metastable equilibrium where the hysteresis persists long enough and is stable enough so that it can be modeled as though it were at equilibrium. The site transformation model of sorption hysteresis is based on the idea that at adsorption equilibrium a fraction of the sites with adsorbed chemical are transformed into sites that reversibly bind the chemical more strongly than the untransformed sites. Therefore both the untransformed and transformed sites are reversible. The model equations makes explicit the transformation step and predict the form of the consecutive desorption isotherm. Depending on the model used for the adsorption isotherm, the model reproduces the Reversible-Resistant and the Linear – Langmuir models that have been fit to experimental adsorption-desorption data. For the site transformation model the form of the consecutive desorption isotherm is not chosen to fit the data but rather follows from the adsorption isotherm and the equation that describes the site transformation. This isolates the hypothesis that produces the hysteresis so that experiments can be designed to test various site transformation mechanisms.

**389 Evaluation of Polycyclic Aromatic Hydrocarbons in Clay Target Fragments and Surface Soil at Shot Gun Ranges** G. Hoeger, B. Magee, ARCADIS. The US Dept of Defense (DoD) oversees more than 3,000 active small arms firing ranges (SAFRs), and there are an additional 9,000 nonmilitary outdoor ranges in the United States. Many of these SAFR sites include trap and skeet ranges, which have elevated lead and polycyclic aromatic hydrocarbon (PAH) concentrations in surface soil in relatively predictable fallout areas based on trap and/or skeet shooting patterns. The clay targets used at trap and skeet ranges are typically made of lime with either coal tar or petroleum pitch as a binding agent. The coal tar and petroleum pitch are the source of PAHs in the target fragments, and they typically include mixtures of high-molecular weight PAHs, many of which are classified as probable human carcinogens by the United States Environmental Protection Agency (USEPA). In 2010, USEPA also proposed to expand the list of PAHs that must be evaluated as potentially carcinogenic to humans. Limited information is available concerning the bioavailability of PAHs in the limestone/coal tar matrix of the clay targets. The work performed by

Baer et al. (1995), who reported that PAHs in the limestone/coal tar matrix were not toxic to aquatic receptors using an EPA toxicity testing methodology, has been used to support the conclusion that PAHs in clay targets are inert and not available for absorption by human or ecological receptors. However, to date, no studies have been identified that directly address the potential bioavailability of PAHs in clay target fragments and impacted surface soil in animal models or human populations. As a result, PAHs in surface soil have become an increasingly important issue for closure of shot gun range (SGR) sites in various states across the country. Many regulators have required the use of default, generic risk-based screening and cleanup levels for the characterization and remediation of SGR sites. These screening and cleanup levels do not account for the limestone/coal tar matrix of the clay targets and assume 100% relative bioavailability. Even when site-specific risk assessment is performed, regulators typically require an assumption of 100% relative bioavailability. The application of default criteria and default risk assessment methodologies at SGR sites, therefore, overestimates the true risks posed by these clay target fragments. This paper will discuss the role that site-specific bioavailability studies can play in developing realistic risk estimates for SGR sites.

**390 Update on Toxicology of 1,4-Dioxane** B.J. Locey, ARCADIS, Risk Assessment and Environmental Services; B. Magee, ARCADIS 1,4-dioxane (1,4-D) was used by the US Dept of Defense (DoD) as a stabilizer for chlorinated solvents used as degreasing agents and in the manufacture of small arms ammunition. Because of its solubility and persistence in groundwater, it is found in and around US Air Force bases, and regulatory authorities are requiring the USAF to monitor for and remediate it. Two recent inhalation bioassays have confirmed 1,4-D's carcinogenic potential in rats by this route, but the relevance of the rat data to human risk assessment is unclear. Based on information provided in USEPA's Integrated Risk Information System (IRIS) Track, inhalation toxicity values have been developed that are currently undergoing interagency review and the draft assessment is scheduled to be released for external peer review during the summer of 2011. It is anticipated that the inhalation toxicity values will have major implications for the investigation and remediation of USAF facilities. This paper will present the key issues associated with the development of inhalation toxicity values for 1,4-D based on the recent and other studies, and will include a discussion of mode of action. The oral toxicological assessment, which was finalized in 2010, and the anticipated draft inhalation assessment, which is scheduled to be available by the end of the summer, will be reviewed in light of the National Academy of Science's recent evaluation of the methods and procedures used to evaluate animal toxicology studies and their appropriateness for deriving toxicity values for use in human health risk assessments in their recent review of the draft IRIS toxicological assessment for formaldehyde, as well as within the context of the on-going debate about the relevance of rodent tumor data for naphthalene to human risk assessment.

### 391 Effectiveness and Effects of Sea Surface Dispersants Used During the Deepwater Horizon Oil Spill and Their Future in Spill Response

**A.C. Bejarano**, Research Planning Inc., Research Planning Inc.; A.J. Mearns, NOAA, Hazmat; J. Farr, E. Levine, NOAA; S. Miles, Louisiana State Univ; J. Michel, Research Planning, Inc. Despite decades of research on dispersants there remains controversy over the effectiveness and efficacy of their use. During the Deepwater Horizon (DWH) oil spill, ~25,505 bbls of dispersants were applied over ~305 square miles during 83 days of continuous aerial operations. A comprehensive evaluation of the dispersant monitoring programs and scientific studies conducted during the DWH oil spill is an important step towards building the foundation for planning and decision making in future spills. One of such programs, Special Monitoring of Applied Response Technologies (SMART), was used to monitor dispersant operations of surface dispersant applications. Tiers of this monitoring effort collected water samples for chemical analysis (~150 samples) and fluorometry measurements (indicators of dispersant effectiveness) at 1 and 10 m depth before and shortly after surface dispersant applications. Chemical analysis included a suite of PAHs (47 analytes in samples from the M/V International Peace and 38 analytes in samples from the M/V Warrior dispersant missions) and TPH measurements, as well as dipropylene glycol n-butyl ether (DPnB; a chemical marker for the presence of dispersants). In selected samples, the toxicity of PAH mixtures to aquatic receptors was characterized using the Equilibrium Partitioning Benchmark Toxic Unit approach (ESBTU), where ESBTU greater than 1 may indicate the potential for adverse effects to aquatic organisms. Preliminary results showed that, despite the lack of a clear correlation between dispersant effectiveness and total TPH concentrations, samples collected at 1 m depth and categorized as chemically dispersed generally had higher TPH concentrations ( $345 \pm 637$   $\mu\text{g/L}$ ) than either naturally dispersed ( $111 \pm 107$   $\mu\text{g/L}$ ) or background samples ( $175 \pm 132$   $\mu\text{g/L}$ ). ESBTUs >1 were observed in water samples collected at the surface (1 m), but not in deeper waters (10 m), and samples with the highest measured DPnB concentrations (>24  $\mu\text{g/L}$ ) had ESBTUs >1 (range: 3-11). In samples with ESBTUs >1, the analytes with the greatest contribution to the overall risk (74-83% to the ESBTU values) included the C1-C4 Chrysene alkylated analogs, C2-C4 Phenanthrenes/Anthracenes, and C2-C3 Fluoranthenes/Pyrenes. Although there was not a clear correlation between ESBTU values and dispersant effectiveness (assessed on-site by SMART teams), ESBTU >1 generally coincided with dispersant applications assessed as moderate to very effective.

### 392 Defining the Proper Role of Toxicity Monitoring in Oil Spill Response Activities

**G.M. Coelho**, D.V. Aurand, J.R. Clark, Ecosystem Management & Associates, Inc. During the Deepwater Horizon oil release, subsea dispersant injection was utilized as part of an overall spill response strategy. BP was directed to develop and implement a water column monitoring program which included shipboard toxicity testing. The commercial aquatic toxicity screening kit (Rotokit M) that utilizes the rotifer, *Brachionus plicatilis*, was selected based on results of prior monitoring work to evaluate shipboard toxicity testing. Even though prior studies indicated that any of the tests considered for shipboard work were difficult to implement, this test had the greatest likelihood of success for this effort. The criteria developed for inclusion in the Directive were arbitrary, and were made without specific data on the organism's sensitivity to crude oil. As expected, when weather conditions were not good, shipboard conditions were not optimal for culture and testing the animals, leading to decreased control survival. However, as they gained experience, technicians were able to achieve acceptable results. Rotifer mortality was never sufficiently high to initiate discussions about restricting injection. In 99% of the 900 samples tested there was 75% or greater survival relative to controls. Concurrently with the field monitoring, laboratory toxicity testing indicated that the 24-hr LC50 ranged from 7.6 to 16.9 ppm. The fact that mortality in the shipboard tests was low is a positive result, but had there been significant mortality it is unclear how that information would have been used in making risk decisions. In short, there is little to recommend such tests, and many reasons to question their utility as a decision tool during emergency response operations. Estimates of hydrocarbon exposure concentrations, initially determined with a CDOM fluorometer and later using shipboard GC MS units, provide a better basis to assess environmental risk than shipboard test results for one species with responses to a variety of shipboard stresses. Field hydrocarbon concentrations can be compared to laboratory results for multiple species and other oils. Even so, neither approach, by itself, can be used to evaluate the appropriateness of dispersant use. While these data are useful, they must

be tied back to Net Environmental Benefit Analysis or Ecological Risk Assessments to reach management decisions. These need to be prepared as part of the planning process, and then reexamined during the spill to determine if the approach still appears to be valid.

### 392.5 Biomarkers of Sustained Exposure in Resident Marsh Fish from Oiled Locations in Barataria Bay, Louisiana Following the Deepwater Horizon Oil Spill

**B. Dubansky**, Louisiana State Univ, Dept of Biological Sciences; C. Rice, Clemson Univ, Biological Sciences; C. Bodinier, W. Stickle, A. Whitehead, F. Galvez, Louisiana State Univ, Dept of Biological Sciences. Coastal marsh habitats in Barataria Bay Louisiana were highly oiled in the months following the Deepwater Horizon Oil spill, and are still contaminated despite extensive cleaning efforts. We are currently investigating the effects of crude oil exposure on resident populations of *Fundulus grandis* (gulf killifish) collected from 15 locations, consisting of oiled and reference sites. Gulf killifish are an ideal species to investigate the effects of exposure to crude oil and its constituents because of their high home-range fidelity, and because they comprise a large percentage of the high marsh biomass and secondary productivity. We utilized immunohistochemistry and ELISA techniques to evaluate the induction of cytochrome p4501A (CYP1A) as a biomarker of exposure to PAHs acting through the aryl hydrocarbon receptor (AhR). Liver, gills, intestine, and head kidneys were sampled in situ and analyzed under laboratory conditions. Results were then paired with water chemistry analyses using passive sampling (polyethylene membrane devices, PEMDs) membranes deployed in Barataria Bay prior to, and at regular intervals following the spill to evaluate temporal changes in total polyaromatic hydrocarbon (PAH) concentration and composition. PAHs attributed to the Deepwater Horizon event peaked in September, and continued at lower levels near affected sampling sites. We found very little CYP1A expression in liver tissues after September, whereas CYP1A protein expression remained highly elevated in the gill, intestine, and renal endothelial cells. Future studies to evaluate the remediation of these areas will include extensive tissue analysis including continued monitoring for CYP1A protein expression, measures of population fitness and fecundity, and continued monitoring of water and sediment chemistry.

### 393 Overview of the Trustee's Aquatic Toxicity Testing Program in Support of the Deepwater Horizon Oil Spill Natural Resource Damage Assessment

**J.M. Morris**, J. Holmes, J. Lipton, Stratus Consulting; M. Gielazyn, R. Ricker, L. DiPinto, NOAA. The Trustees for the Deepwater Horizon natural resource damage assessment have developed a comprehensive aquatic toxicity testing program to assess the potential impacts of Deepwater Horizon oil and dispersant on a variety of Gulf of Mexico fish and invertebrates. NOAA and Stratus Consulting are co-leading the program, which includes scientists from multiple federal, state and private laboratories and research institutions. The Deepwater Horizon incident resulted in oil releases to many different habitats under many different conditions over a long period of time. Organisms may have been exposed to wide range of oil variations, from relatively fresh oil and dispersant in pelagic habitat near the well, to highly weathered oil in shallow water or shoreline habitats. Therefore, the Trustees are exposing a wide variety of organisms to multiple oil preparations under different conditions. Example species in the toxicity testing program include pelagic fish such as cobia (*Rachycentron canadum*) and mahi mahi (*Coryphaena hippurus*); shallow water fish such as speckled sea trout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus*); typical model fish such as sheepshead minnow (*Cyprinodon variegatus*); motile invertebrates such as white shrimp (*Litopenaeus setiferus*) and blue crab (*Callinectes sapidus*); and sessile invertebrates such as eastern oysters (*Crassostrea virginica*). The testing program includes exposing organisms to unweathered source oil, artificially weathered source oil, surface slick oil collected near the Macondo well, and/or more weathered slick oil collected closer to shore. Various methods will be employed to create water-accommodated fractions (WAFs) of oil, including high-energy mixtures, low-energy mixtures, and chemically-enhanced WAFs (CEWAFs) using COREXIT 9500 dispersant. The Trustees will evaluate several variables that may affect toxicity, such as potential enhancement of PAH toxicity under ultraviolet light, or changes in toxicity resulting from changes in salinity. Multiple toxicological endpoints will be examined in addition to mortality, including reduced fecundity/reproductive success, adverse changes to cardiac and metabolic functions, and reduced predator avoidance. This comprehensive aquatic toxicity program will help the Trustees to evaluate the adverse effects of the Deepwater Horizon oil and dispersant on exposed fish and invertebrates in the Gulf of Mexico.



**394 Mixture Toxicity of Louisiana Sweet Crude Oil and Corexit 9500 to Estuarine Organisms** M.E. DeLorenzo, NOAA, National Ocean Service, Dept of Marine Ecotoxicology, NOAA, National Ocean Service; K.W. Chung, P.B. Key, NOAA, National Ocean Service; L. Ferguson, Duke Univ, Dept of Civil and Environmental Engineering; M.H. Fulton, NOAA, National Ocean Service. About 2 million gallons of dispersants were applied to the oil spilled in the Deepwater Horizon event. Here we describe the results of laboratory testing aimed at quantifying the interactive effects of oil and dispersants to estuarine species. The toxicity of Louisiana Sweet Crude Oil to four representative estuarine species (fish, *Fundulus heteroclitus*; grass shrimp, *Palaemonetes pugio*; juvenile clam, *Mercenaria mercenaria*; green algae, *Dunaliella tertiolecta*) was determined with and without the addition of the oil dispersant, Corexit 9500. For each species, a static, 96 hour laboratory exposure was conducted at 25°C, 16h light:8h dark photoperiod, and 20 ppt salinity. The oil was added as a surface slick, and then Corexit was applied (1:20 v/v). Mortality was assessed daily. Growth rate was determined for the algae, and EROD activity was determined from the surviving fish after 96h. Oil toxicity varied among the species tested, with grass shrimp being the most sensitive. The mixture of Corexit 9500 with Louisiana Sweet Crude Oil, however, was significantly more toxic than the oil alone. For example, with grass shrimp, a 1:20 application of Corexit to the oil treatments increased mortality by approximately 60% compared to the same concentrations of oil alone. Chemical analysis of the oil and the Corexit 9500 was performed to quantify oil levels in the water after 96h, and to examine degradation of the dispersant over time. Mortality effects levels for the individual compounds and the mixture will be presented, along with results for the sublethal endpoints and chemical fate analysis.

**396 Monitoring Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood in Mississippi in Response to the Gulf Oil Spill** K. Armbrust, Office of the State Chemist – Mississippi, Mississippi State Univ; K. Xia, Office of the State Chemist – Mississippi, Mississippi State Chemical Lab; G. Hagood, Office of the State Chemist – Mississippi, Mississippi State Univ; J. Jewell, D. Diaz, Mississippi Dept of Marine Resources; A. Brown, Mississippi State Univ, Biochemistry, Molecular Biology, Entomology and Plant Pathology; N. Gatian, H. Folmer, Mississippi Dept of Environmental Quality. Following the sinking of the Deepwater Horizon, the State of Mississippi began sampling and monitoring crabs, shrimp, oysters and several species of fish from numerous locations within Mississippi State Waters. From the end of May 2010 to date, over 400 samples have been analyzed by the State for Polycyclic Aromatic Hydrocarbons (PAHs) as listed in the NOAA method for analysis of PAHs in seafood. Additional samples were also collected and submitted to the NOAA laboratory in Pascagoula, MS to support the reopening of state waters in accordance with the protocol jointly developed by the gulf coast states, FDA and NOAA. PAHs have not been detected in any sample collected to date at levels above the Level of Concern (LOC) as established in the reopening protocol. However PAHs were routinely detected in most samples at low part-per-billion levels and are consistent with values commonly detected in samples measured in other studies along the gulf coast before, and unrelated to, the oil spill. The levels measured in seafood were also consistent with or below levels of PAHs detected in food items (smoked turkey, ham, chicken, catfish and barbecued pork) purchased at major retail supermarkets and restaurants.

**397 Mapping of Sediment and Benthic Conditions around the Deepwater Horizon Accident Site** J. Germano, D. Browning, Germano & Associates, Inc.; E. Revelas, Integral Consulting; L. Riege, Cardno Entrix. In Spring 2011, as part of a cooperative effort by BP and Federal Trustees to assess the potential effects of the Deepwater Horizon accident on the natural resources of the deep benthic sediments, a study team mapped the extent of deposits that may have resulted from drilling and well control activities in the area surrounding the MC-252 wellhead. The study used a Sediment Profiling and Plan View Imaging (SPI/PV) camera system to collect both surface and cross-sectional images of seafloor sediments in approximately 1,500 meters of water. The study complements previous and planned studies of sediment PAH concentrations and benthic infaunal community structure in the area around the wellhead. Comparison studies include (1) historical studies conducted by the Bureau of Ocean Energy Management, Regulation and Enforcement (BOEMRE) (2) recent studies by the Unified Area Command Operational Science Advisory Team (OSAT), and (3) a follow-up study by BP and the Federal Trustees in Spring 2011. The talk presents the results of the SPI/PV study and correlations between sediment PAH

concentrations published by the OSAT and by the historical BOEMRE studies. The talk also discusses planned analyses correlating the SPI/PV results with infaunal community and sediment chemistry results from the Spring 2011 follow-up study.

**398 Impact of Global Climate Change on Foundations of Environmental Toxicology and Chemistry: Results of the Pellston Workshop** R.G. Stahl, DuPont Company, Corporate Remediation Dept, Principal Consultant, DuPont Corporate Remediation Group. A SETAC, Pellston-style workshop was held July 16-21, 2011 at the Wingspread Conference Center, Racine, Wisconsin to address the potential influence of global climate change (GCC) on the foundations of environmental toxicology and chemistry. Several years in the making, the workshop culminated the efforts of scientists from around the world to determine what, if any, changes need to be made in the underlying approaches used to assess the potential fate and effects of chemical toxicants. In addition, the participants addressed the potential that GCC will have on environmental restoration projects and how the design and implementation of those projects may need to change. A summary of the workshop proceedings will be presented along with plans for final publication of the workshop results.

**399 Climate Change Impacts on Exposures, Fate, Transport and Deposition of Chemicals in the Environment** T. Gouin, Unilever, Safety and Environmental Assurance Centre; J. Armitage, Univ of Toronto Scarborough; I. Cousins, Stockholm Univ; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute; C. Ng, ETH. A SETAC Pellston workshop, held in July, 2011, brought together an international group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment, to advance the level of scientific understanding of how global climate change may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by climate change. The emphasis of workgroup 1 was aimed at assessing the influence of climate change on exposure, fate, transport, and deposition of chemicals in the environment, and how potential changes may impact environmental risk and hazard assessments. A key challenge identified relates to the complexity of interconnected environmental processes that may result in enhanced or reduced exposure of chemical contaminants. The complexity of the interactions can lead to non-intuitive results, making projections of how chemical fate and bioavailability might change in a world impacted by GCC difficult to assess. Nevertheless, efforts are made to demonstrate how models can quantify the physical-chemical properties of substances that may be more sensitive to changes in climate than others. Additionally, we attempt to demonstrate which global regions enhanced chemical exposure is more likely to occur. Utilizing an appreciation for the uncertainty and sensitivity of model parameters, as defined for both environmental fate models and climate change models, we attempt to demonstrate how model output can complement the collection of empirical data in a manner that can better inform our ability to assess potential relationships between chemical fate and bioavailability and changes in climate that can be attributed to climate change.

**400 Mechanistic Toxicology in the Face of Global Climate Change** M.J. Hooper, USGS, Columbia Environmental Research Center; G. Ankley, USEPA; D.A. Cristol, College of William and Mary; L.A. Maryoung, University of California, Riverside; P.D. Noyes, Duke Univ, Nicholas School of the Environment; K.E. Pinkerton, University of California, Davis, Center for Health and the Environment. Incorporation of global climate change (GCC) effects into regulatory assessments of chemical risk and injury requires an understanding of GCC effects on mechanisms of chemical toxicity. Effects on mechanisms include those acting on toxicokinetics of chemical absorption, distribution, metabolism and excretion, as well as those affecting toxicodynamic interactions between chemicals and target molecules. GCC can further modify an organism's baseline physiological processes for coping with the external environment (water balance, thermoregulation, immune-endocrine-neurological systems), processes with distinct age, sex, and taxonomic specificities. In organisms living in GCC modified climates, increased investment in adaptation to GCC can lead to increased susceptibility to chemicals, disease and other stressors. Physiological processes critical to surviving temperature, hydration, nutrition and disease stresses will be particularly sensitive to even the slightest perturbations by chemicals when pushed to their functional limits. In simplest terms, GCC can make

organisms more sensitive to chemical stressors while, alternatively, chemical exposure can make organisms more sensitive to GCC stressors. Implications of GCC interactions with chemical mechanisms of toxicity are applicable to both ecological and human health effects assessments. To better address the complexities of these interactions, we employed adverse outcome pathways (AOPs), constructs that depict linkages between molecular initiating events and subsequent responses occurring across biological levels of organization, culminating in impacts in individuals or populations that can be used for assessing risk. Through a series of examples and case studies, we demonstrate how chemical- and climate-specific exposure scenarios can lead to adverse outcomes. Scenarios are prospective, hypothesizing outcomes based on known or anticipated chemical/GCC interactions, as well as retrospective, where mechanisms are proposed for known or demonstrated chemical-climate interactions. Understanding GCC interactions along AOPs provides opportunities for extrapolation between species or other levels of organization, development of questions, hypotheses and focal areas for further research, and improved inputs for risk and injury assessments.

**401 Combined Effects of Climate Change and Toxicants on Populations and Communities** J. Moe, Norwegian Institute for Water Research (NIVA), Climate and environmental modelling, Norwegian Institute for Water Resea, Section for Freshwater Biodiversity; K.A. De Schampelaere, Ghent Univ, Environmental Toxicology and Aquatic Ecology, Ghent. Univ (UGent), Environmental Toxicology and Aquatic Ecology, Ghent Univ, Environmental Toxicology and Aquatic Ecology; W.H. Clements, Colorado State Univ, Fish, Wildlife and Conservation Biology, Colorado State Univ, Colorado State Univ, Fish, Wildlife and Conservation Biology; M. Sorensen, Environ International, ENVIRON International Corporation, Senior Science Advisor, Environ International; P.J. van den Brink, Alterra and Wageningen Univ, Alterra and Wageningen Univ, Centre of Water and Climate, Alterra and Wageningen Univ; M. Liess, Helmholtz centre for environmental research – UFZ, Dept of System Ecotoxicology, UFZ Center for Environmental Research, Dept of Chemical Ecotoxicology, Helmholtz centre for environmental research – UFZ, Dept of System Ecotoxicology. A SETAC international workshop, held in July, 2011, brought together a group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment and natural resource damage assessment, to advance the level of scientific understanding of how global climate change (GCC) may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by GCC. This presentation will discuss the anticipated joint impacts of GCC and toxicants on higher levels of ecological organization, i.e., populations and communities. Climatic changes such as higher temperature and CO<sub>2</sub> levels have documented negative impacts on many species, e.g., amphibians and coral reefs. GCC-related stress and toxicant exposure can therefore be regarded as multiple stressors, where the combined impacts on individuals can be stronger than the sum of the two factors (i.e., synergistic interaction) or weaker than the sum (i.e., antagonistic interaction). Since GCC can also influence the exposure, fate and toxicity of toxicants, the combined impacts of GCC and toxicants may be more complex than for other multiple stressors. Moreover, GCC can also directly impact on population and community processes, e.g., disrupt the timing of predator-prey interactions. A great challenge for ecotoxicology is to predict the how joint effects of GCC and toxicants at the individual level (e.g., reduced survival or reproduction) will be transferred to the population level (e.g., population growth rate) or to the community level (e.g., species richness). Given the large complexity and variability of GCC impacts on the environment, it is not possible to give general predictions for GCC x toxicant interactions on higher ecological levels. In this paper we focus on (i) how GCC directly and indirectly can alter toxicant exposure and impacts on organisms; (ii) species traits that may have particular importance for species' responses to GCC and thus to combined effects of GCC and toxicants, and (iii) ecological and evolutionary mechanisms which may have particular importance for GCC x toxicant interactions across ecological levels. Within this framework, case studies from three continents are used to illustrate the complexity of joint effects of GCC and toxicants on populations and communities.

**402 How Will Global Climate Change Affect Human Health Risk Assessment?** J.M. Balbus, NIEHS; A. Boxall, Univ of York, Environment Dept; R. Fenske, Univ of Washington, Dept Env Occ Health Sciences; T. McKone, Univ of California, Berkeley, Univ of California and Lawrence

Berkeley National Laboratory, Univ of California, Univ of California and Lawrence Berkeley National Laboratory; L. Zeise, California EPA Office of Environmental Health Haza. Global climate change is predicted to alter long-term weather characteristics in different regions. These changes, including increased temperature, greater precipitation extremes, and loss of glacial and polar ice, have implications for human exposure to chemical contaminants. Climate change may also directly and indirectly affect the vulnerability of humans to chemical exposures. Changes in human exposure may arise from altered use, inputs, fate and transport of chemicals due to climatic and other drivers. Human vulnerability may be affected directly by heat and other weather-related stressors, or indirectly through altered co-exposures or disease patterns. To further explore the implications of climate change for the assessment and management of chemical risks, the authors examine four specific risk contexts: natural toxins, pesticides, air pollutants, and legacy chemicals (e.g., mercury, POPs). For the specific types of decisions to be made in each of these contexts, we examine how assessments and management decisions may be affected by climate change, and how significant the impacts of climate change may be. Climate change is likely to both increase and decrease human exposures, depending on the specific contaminant and specific region or other exposure context. There is limited evidence that climate change will increase the sensitivity of humans to chemical exposures. But small changes in exposure variability or human vulnerability can translate into significant changes in population risk profiles. To assess and manage chemical risks effectively, exposure data sources will need to be regularly updated and defaults and assumptions used in exposure assessment evaluated in a context of changing climate. Monitoring and sampling should be done with frequency sufficient to capture variability, which is likely to increase in many places. There are many research gaps in interactions between climate and weather parameters and human responses to chemical exposures. These factors will all exacerbate gaps in chemical protection between developed and developing countries.

**403 Ecological Risk Assessment in the Context of Global Climate Change** W.G. Landis, Institute of Environmental Toxicology, Western Washington Univ, Western Washington Univ, Institute of Environmental Toxicology, Western Washington Univ, Institute of Environmental Tox. & Chem.; J. Durda, Integral Consulting Inc; M. Brooks, Southern Illinois Univ; P.M. Chapman, Golder Associates Ltd; C. Menzie, Exponent, Inc., Exponent, Principal; R.G. Stahl, DuPont Company, Corporate Remediation Dept, Principal Consultant, DuPont Corporate Remediation Group; J. Stauber, CSIRO. This paper originates from the SETAC Pellston Workshop entitled "The Influence of Global Climate Change (GCC) on the Scientific Foundations and Applications of Environmental Toxicology and Chemistry" and specifically, from the workgroup charged with determining how ecological risk assessment (ERA) will encompass the challenges presented by GCC. Although the basic ERA structure remains useful, the process needs to broaden beyond contaminant issues per se to include interactions with changing habitats that alter biologic communities and recognize that landscape ecology (a.k.a. regional risk assessment) needs to be embraced to optimize effective environmental management decisions in the context of GCC. We propose seven principles for conducting ERAs under GCC: 1) evaluate a priori whether climate-related factors are likely to impact to a given ERA process and management decisions; 2) develop and express assessment endpoints as ecosystem services; 3) recognize that responses, (changes in ecosystem services), can be positive or negative; 4) recognize that the risk assessment process requires a multiple stressor approach with non-linear interactions; 5) develop conceptual cause-effect diagrams that consider context-dependent management decisions, at the appropriate spatial and temporal scales while ranking direct and indirect effects; 6) determine the major drivers of uncertainty by estimating and bounding stochastic uncertainty spatially, and across time; and, 7) plan for adaptive management to account for changing environmental conditions and consequent changes to ecosystem services. Given the complexities and uncertainties associated with GCC, good communication is essential for making risk-related information understandable and useful for managers and stakeholders.

**404 Environmental Contaminants and Global Climate Change: Implications for Environmental Damage Assessment and Restoration/Rehabilitation** R.C. Helm, US Fish & Wildlife Service, Chief, Division Environmental Quality, , US Fish & Wildlife Service – Chief, Division Environmental Quality, ; S. Brasfield, US Army Engineer Research and Development Center, Environmental Laboratory; A. Fritz, NOAA/OR&R/

CPRD, Coastal Protection and Restoration Division, NOAA/OR&R/ARD, Assessment and Restoration Division, NOAA/OR&R/CPRD, Coastal Protection and Restoration Division; C. Hickey, Natl. Inst. of Water & Atmospheric Res., NIWA Ecosystems; P. Johnson, Fisheries and Ecological Services, US Fish and Wildlife Service; J. Rohr, Univ of South Florida, Dept of Integrative Biology. A SETAC international workshop, held in July, 2011, brought together an international group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment and natural resource damage assessment, to advance the level of scientific understanding of how global climate change (GCC) may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by GCC. The emphasis of the restoration workgroup was environmental damage assessment and restoration/rehabilitation in relation to GCC, specifically addressing: (i) legislation relating to contaminant management, damage assessment and restoration/rehabilitation requirements; (ii) experience with damage assessment and restoration implementation; and (iii) potential direct and indirect influences of GCC on damage assessment and restoration/rehabilitation processes. An example is provided in the US, where legislated requirements for damage assessment and restoration incorporate chemical exposure and ecosystem effects from site contaminants to assess the injury to resources and estimate damages paid for restoration of those resources. GCC will impact the processes of assessing injury and rehabilitating/restoring and conserving resources by affecting the magnitude of impact of contaminants on natural resources and altering potential restoration/rehabilitation efforts. Baseline/reference conditions for estimating resource injury and restoration/rehabilitation also may shift significantly and exhibit greater variability due to GCC, representing a significant challenge to practitioners. This presentation will discuss how such future environmental damage assessments and restorations might be influenced by GCC and provide recommendations of research needs in the field.

**405 Potential Effects of Climate Change on Water Quality in Mineralized Watersheds** A.S. Todd, US Geological Survey, Crustal Geophysics and Geochemistry Science Center; A. Manning, USGS, Crustal Geophysics and Geochemistry Science Center; D. McKnight, Univ of Colorado at Boulder, Institute of Arctic and Alpine Research; C. Crouch, Institute of Arctic and Alpine Research. A unique long-term water chemistry dataset has been compiled for the Upper Snake River, a stream draining a mineralized mountain watershed in Summit County, Colorado, USA. Trace metal and sulfate concentrations in stream water at baseflow have increased by a factor of two to four over the past three decades. Several small mines are located in the watershed, but they are abandoned and no mining or mine-remediation activities have occurred during this period. A similar increase in sulfate concentration has been observed in another part of the Colorado Rockies in lakes within mineralized watersheds also free of recent mining activity. These trends are concerning because mineralized watersheds are common throughout the mountains of the western US, and further water quality degradation could negatively impact important downstream drinking water resources and aquatic ecosystems. At sites in the lower Snake River watershed, resident fish populations are vulnerable to increasing metals concentrations, as is reflected in reduced densities of brook trout captured through electrofishing surveys in recent years. Climate change is a plausible cause of these observed trends, as surface and sub-surface hydrology play an important role in the generation of acid-rock drainage (ARD). While the specific links between climate and the natural production of low-pH, ARD in mineralized watersheds are poorly understood, preliminary modeling results suggest that water-table depth, recharge rate, and the magnitude of seasonal water-table fluctuations may be of primary importance. Climate change scenarios involving increasing temperature and decreasing snowpack, as predicted for much of the western US, may thus portend further water quality degradation in many mineralized watersheds.

**406 Absorbance and Fluorescence Properties of Natural Organic Matter as Quality Measures Predicting Protective Effect Against Cu Toxicity to *Daphnia magna*** H. Al-Reasi, C. Wood, McMaster Univ, Biology; S. Smith, Wilfrid Laurier Univ, Dept of Chemistry, Wilfrid Laurier Univ, Chemistry. The protection of natural organic matter (NOM) against metal toxicity, in particular copper (Cu), is potentially source-dependent. In the presence of NOM of different sources, but similar quantity (estimated as dissolved organic carbon, DOC, in mg C L<sup>-1</sup>) and water chemistry, observed variability in Cu acute median-lethal concentrations (LC50s) remains

unexplained. Here, quality measures of NOM, based on absorbance and fluorescence measurements, are proposed for possible incorporation into toxicity models, such as biotic ligand model (BLM), in order to improve predictive capabilities and account for observed NOM source-dependent variations. Using *Daphnia magna*, Cu toxicity tests were conducted in dechlorinated hard water (140 mg CaCO<sub>3</sub> L<sup>-1</sup>) in the absence and presence of different freshwater NOMs isolates added at two DOC concentrations. With ranges of dissolved LC50s of 23.4 – 160.1 and 32.0 – 211.5 µg Cu L<sup>-1</sup> reported at 3 and 6 mg DOC L<sup>-1</sup>, respectively, up to 4-fold source-dependent differences in protection was observed. Relative humic-like fluorescent concentration was quantified for each NOM source using parallel factor analysis (PARAFAC), a multivariate approach applied to excitation-emission matrices. Optimization of lethal Cu accumulation (LA50) and utilization of PARAFAC-derived %HA as BLM input instead of the default 10%HA significantly improved the prediction of the BLM. At LA50 of 0.0120, the predicted and observed LC50s were weakly correlated (predictive LC50 = 0.41 measured LC50 + 17.04, R<sup>2</sup> = 0.28) with the use of default 10% assumption, however the relationship turned strong (predictive LC50 = 1.00 measured LC50 + 4.16, R<sup>2</sup> = 0.77) with the use of PARAFAC-derived %HA. The specific absorbance coefficient (SAC<sub>340nm</sub>), an easily measured index of aromaticity of NOM, was highly correlated (R<sup>2</sup> = 0.96) with the PARAFAC-derived %HA of the NOMs, suggesting the contribution of aromatic constituents to both fluorescence and absorbance. To facilitate incorporation into the BLM, SAC<sub>340nm</sub> for each NOM isolate was employed to predict %HA, which was optimized separately for each LC50 determination. The optimized PARAFAC-derived %HA were still strongly linked to the SAC<sub>340nm</sub> (R<sup>2</sup> = 0.80), indicating the applicability of the BLM to have fluorescence quality measure with absorbance based correction. Funded by NSERC to CMW and DSS, and scholarship from Government of the Sultanate of Oman to HAA.

**407 Biofilm Response to Metal-contaminated Sediment Depends on Sediment and Stream Context** D. Costello, A. Burton, Univ of Michigan, School of Natural Resources & Environment. In metal-contaminated streams, biofilms are exposed to and accumulate metals, which can reduce biofilm growth, alter function, and expose consumers to potentially toxic dietary metal. Exposure can occur through dissolved metal in surface waters; however, metal-contaminated sediments may also affect biofilms. We exposed biofilm to metal-spiked sediments (Ni and Cu together) from two streams by growing it on porous glass disks atop sediment-filled cups. Biofilm cups were placed in closed (Big Pup) and open (Salmon-Trout) canopy streams near Marquette, MI. Two sediments, which were characterized by acid-volatile sulfide, organic carbon, and iron and manganese oxide content, were amended with metal and deployed in each of the streams. After 28 days, biofilms were analyzed for O<sub>2</sub> production, chlorophyll *a*, biomass, and metal concentrations. Biofilm production and chlorophyll *a* were sensitive to metal concentrations, but the response differed in the two streams; biofilm cups placed in the closed canopy stream were more sensitive to metals than biofilms in the open stream. Geochemistry differed between our two sediments, yet the biofilm growth and production was reduced more when grown on sediments with greater concentrations of binding ligands. This suggests that current metal bioavailability models may not be amenable to natural biofilm communities and that photosynthetic activity may modify the toxicity of contaminated sediments.

**408 Copper-induced Oxidative Stress in the Sea Anemone *Actinia bermudensis*** V. Cavicchioli-Azevedo, M.M. Lauer, N.L. Yano, A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. Copper catalyzes the formation of reactive oxygen species (ROS), but little is known about its effect on antioxidant defenses and protein repair mechanism in osmoconformers. We evaluated the copper effects on antioxidant defenses [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), glutamate cysteine  $\gamma$ -ligase (GCL), glutathione-S-transferase (GST)], total antioxidant capacity against peroxyl radical (ACAP), oxidative damage (lipid peroxidation and protein carbonyl content), and protein repair mechanism (HSP70) in the sea anemone *Actinia bermudensis*. Animals (n=5) were exposed to copper (5, 10, 50 µg Cu/L) for 96 h at salinity 30 ppt. A control treatment (no copper addition) was also run. Temperature (20°C) and photoperiod (12h L:12h D) were fixed. No mortality was observed. Copper exposure increased SOD activity, probably due to an increased anion superoxide formation. Copper also increased CAT activity, likely associated with a higher H<sub>2</sub>O<sub>2</sub> concentration



resulting from the increased SOD activity. Since SOD and CAT represent the first line of defense against the anion superoxide and  $H_2O_2$ , their increased activity protected against ROS. In the other hand, GPx activity was inhibited in copper-exposed animals, decreasing the efficiency of the second line of defense against ROS. In addition, no effect on GSH level, GCL and GST activity was observed. This lack of effect was probably due to the reduced use of GSH by GPx. After copper exposure, a reduced ACAP was observed, likely due to the inhibition of GPx and the low consumption of GSH. Reduced ACAP led to an increased lipid peroxidation and protein carbonyl content in copper-exposed sea anemones. An increased HSP70 concentration was observed in sea anemones exposed to 5 and 10  $\mu\text{g Cu/L}$ , suggesting a stimulation of the mechanism of protein repair. At 50  $\mu\text{g Cu/L}$ , this mechanism seems to be overwhelmed, since a decrease in the HSP70 concentration was observed. Taken altogether, results showed that copper exposure reduces the antioxidant capacity leading to oxidative damage in lipids and proteins in spite of an increased response of the protein repair mechanism in the sea anemone *A. bermudensis*. These findings indicate that the mechanism of copper toxicity in marine osmoconformers is associated with oxidative stress. [Supported by the Brazilian CNPq (INCT-TA) and CAPES (Ciências do Mar) and the Canadian IDRC].

**409 Cu and Pb Internalization by *Chlamydomonas reinhardtii* at Varying Carbonate Concentrations: Agreement with FIAM?** P. Sanchez-Marin, C. Fortin, P.G. Campbell, Institut National de la Recherche Scientifique, Centre Eau Terre Environnement. Over the past 10+ years, substantial efforts have been devoted to the development of biotic ligand models (BLMs) to predict the bioavailability of metals. These models are focused mainly on the responses of metal bioavailability (uptake or toxicity) to variations in major ion composition ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ), pH, and to the presence of natural ligands such as dissolved organic matter. However, the effect of carbonate, an inorganic ligand ubiquitously present in aquatic environments, has rarely been investigated. Carbonate concentrations can vary in natural waters due to differences in the underlying geology, pH variations, as a result of photosynthesis/respiration processes, or due to the continuously increasing anthropogenic  $\text{CO}_2$  concentrations in the atmosphere. Both carbonate and bicarbonate can form complexes with metal ions, and although the stability constants of these complexes are quite well established for Cu, a high uncertainty remains on those defining the degree of complexation of Pb with these anions. By dissolving  $\text{NaHCO}_3$  in closed vessels and accurately measuring inorganic carbon concentrations, we have studied both the speciation and bioavailability of Cu and Pb at varying carbonate concentrations (from 10 to 3000  $\mu\text{M C}$ ) at constant pH and ionic strength.  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  concentrations were measured by ion selective electrodes and related to the short term internalization of Cu and Pb by the freshwater microalga *Chlamydomonas reinhardtii*. The results showed that the internalization of both metals depends on the activity of the free metal ions, as controlled by their complexation with carbonate. There was no evidence for uptake of metal carbonate complexes. These results can be included in current BLMs for freshwater algae to take into account the presence and variation of dissolved inorganic carbon concentrations in natural waters.

**410 Influence of Water Quality to Zinc Toxicity in the Florida Apple Snail (*Pomacea paludosa*)** T.C. Hoang, Florida International Univ, Southeast Environmental Research Center/Dept of Environ Study, Loyola Univ Chicago, Center for Urban Environmental Research and Policy; G. Rand, Florida International Univ, Ecotoxicology and Risk Assessment. Aquatic toxicology research has been conducted to characterize the toxicity of zinc to standard aquatic organisms (e.g., Fathead minnow, *Daphnia magna*, etc.). However, research of zinc toxicity to non-standard test organisms such as Apple snails has not been conducted. Apple snails are widely distributed in the southeastern US. In Florida, apple snails play a major role as the main food source of many higher trophic organisms in the Everglades ecosystem. Our recent research indicates that the Florida apple snail is sensitive to copper and water quality significantly influences Cu toxicity to Florida apple snails. The present research characterizes the influence of water quality on Zn toxicity to Florida apple snails. Static renewal acute toxicity tests were conducted in this study. Water quality used in this study varied from soft to very hard water. Concentrations of dissolved organic carbon (DOC) ranged from 0 to 10 mg/L. Results of this study indicate that hardness had a protective effect on zinc toxicity to Florida apple snails. The 48-h LC50 values of the snail increased from 160 to 1190  $\mu\text{g/L}$  dissolved Zn when hardness increased from 20 to 200 mg/L, as  $\text{CaCO}_3$ . The 96-h LC50 values were 140

$\mu\text{g/L}$  and 1017  $\mu\text{g/L}$  dissolved Zn at hardness of 20 mg/L and 200 mg/L as  $\text{CaCO}_3$ , respectively. These results are in contrast with the results of our earlier study that hardness was not protective to Florida apple snails from Cu toxicity. Similar to Cu, DOC also mitigated the toxicity of Zn to Florida apple snails. When DOC increased from 0 to 10 mg/L, 48-h and 96-h LC50 values of the snail increased from 605 to 845  $\mu\text{g/L}$  and from 463  $\mu\text{g/L}$  to 632  $\mu\text{g/L}$  dissolved Zn, respectively. Results of this study indicate that Florida apple snails are sensitive to Zn. The effects of pH and alkalinity on Zn toxicity to Florida apple snails should be investigated and a biotic ligand model for Zn and apple snails should be developed.

**411 Modelling Uptake of Trivalent Trace Metals by Phytoplankton Using a BLM Approach** A. Cremazy, P. Campbell, C. Fortin, INRS-ETE. Major progress has been achieved in recent years in our ability to predict metal bioavailability to aquatic biota. This is due in large measure to the development and improvement of models such as the Biotic Ligand Model (BLM). These models allow for the prediction of trace metal uptake and toxicity to aquatic organisms in natural waters and are thus useful tools for the ecological risk assessment of metals. According to the BLM, metal accumulation, and therefore toxicity, are proportional to the free ion activity. The applicability of the BLM to divalent trace metals has been thoroughly investigated since the pioneering work of Sunda and co-workers in the 1970s. However, its relevance to trivalent trace metals such as aluminium is still largely unknown. Although the toxic effects of aluminium are relatively well documented, the uptake mechanisms are still speculative and the links between speciation and accumulation remain elusive. This situation is mainly caused by the analytical difficulties encountered when working with trivalent metals: their aqueous coordination chemistry is complex, they tend to bind tightly to cell walls, and there is a lack of affordable and suitable radio-isotopes and a dearth of sensitive analytical techniques. Scandium was used in the present study as a substitute for aluminium, to investigate the applicability of the BLM to unicellular algae. Scandium is a trivalent trace metal with chemical similarities to aluminium and it has a suitable radio-isotope ( $\text{Sc-46}$ , half life of 83.8 days) for carrying out uptake experiments. Measurements of scandium internalization fluxes ( $J_{\text{int}}$ ) in a unicellular alga (*Chlamydomonas reinhardtii*) have been performed in kinetic uptake experiments. Changes in the chemistry of the exposure media ([Sc], pH, ionic strength, etc.) were then related to the  $J_{\text{int}}$  values in order to explore the uptake mechanisms of scandium and to identify which metallic species are involved in the internalization. For example,  $J_{\text{int}}$  increased from  $3.0 \pm 0.1$  to  $31.6 \pm 3.1 \text{ nmol}\cdot\text{m}^{-2}\cdot\text{nM}^{-1}\cdot\text{h}^{-1}$  ( $n=3$ ) with a decrease in the pH from 7.75 to 6.00.  $J_{\text{int}}$  also increased with a decrease in  $\text{Ca}^{2+}$  and  $\text{Na}^+$  concentrations. The ability of the BLM to explain the uptake kinetics will be discussed.

**412 The Effect of Cd on Repeated Swim Performance in Relation to Metabolic Recovery in Rainbow Trout (*O. mykiss*) During Sublethal Waterborne Exposure** J. Cunningham, Wilfrid Laurier Univ, Biology; J. McGeer, Wilfrid Laurier Univ. Swimming ability is frequently used to characterize the integrated metabolic costs associated with exposure to contaminants, including Cd. Repeat swimming challenges provide a more sensitive indicator of effects by identifying impairment that was not observed in a singular swim challenge alone. Sublethal Cd exposure has previously been shown not to affect swimming performance in individual swim challenges; however we have recently shown that exposure to 2  $\mu\text{g Cd L}^{-1}$  resulted in a dramatic reduction in performance in subsequent swim trials. For example, performance in the second swim trial, following an initial swim and 30 min recovery period, resulted in reductions of sustained swimming capacity (Ucrit) as great as 31% in Cd exposed brown trout (*Salmo trutta*). Drawing on this, recovery periods of varying duration were used in order to determine the role of the rest period in the recovery of swimming ability and metabolic parameters. This study employs two consecutive swimming challenges, separated by a recovery period of varying duration (0.5, 1.5 and 6 h) in order to investigate the effects of Cd (2  $\mu\text{g Cd L}^{-1}$ , 140 mg  $\text{CaCO}_3$ ) on repeated swimming ability in juvenile rainbow trout (*Oncorhynchus mykiss*). Blood, organ and muscle samples were taken before and after each of the swim trials and rest periods, and tissue Cd burden, plasma ion composition, metabolic fluctuations and stress response were measured. Following a 30 minute recovery period, Cd exposed fish had a decreased Ucrit of  $3.0 \pm 0.3 \text{ bl s}^{-1}$ , compared with  $4.4 \pm 0.3 \text{ bl s}^{-1}$  in controls, in subsequent challenges. However, with a 1.5 hour recovery period Cd exposed fish performed significantly better in subsequent swimming challenges ( $5.2 \pm 0.2 \text{ bl s}^{-1}$ ) than did controls ( $4.3 \pm 0.2 \text{ bl s}^{-1}$ ), with no differences observed

between treatments after 6 hours of recovery. Metabolic fuel sources, utilized to power muscular contraction and physical activity, such as glycogen and ATP were directly linked to the changes in swim performance at different recovery times. The increased swimming capacity of Cd exposed fish following a 1.5 h recovery was correlated with greater restoration of glycogen (5x) and ATP (1.5x) compared to controls. The results establish linkages between exposure, bioaccumulation and the physiological disruption induced by Cd to provide an improved understanding of the mechanisms underlying impairment of whole animal performance. This research is supported through the NSERC Discovery Program.

**413 Toxicity of Metal Mixtures to *Daphnia magna*: Implications for a Multi-metal, Multi-site Biotic Ligand Model** L. Meyer, Arcadis US, Inc., ARCADIS; J. Ranville, Colorado School of Mines, Chemistry and Geochemistry, Colorado School of Mines; M. Pontasch, Colorado School of Mines; R. Santore, A.C. Ryan, HDR|HydroQual; J. Gorsuch, Copper Development Association; W.J. Adams, Rio Tinto, Product Stewardship. For decades, a simplistic summation of toxic units based on concentrations of dissolved metals has been used to semi-quantitatively predict the toxicity of metal mixtures. However, that approach tends to over-predict toxicity. In its place, we have been developing a mechanistic model based on tissue residues of metals and on the concept that the toxicity of a mixture of metals can be either dose-additive or response-additive, depending on the mechanisms of action. To calculate tissue residues and thereby predict toxicity across wide ranges of water quality, we have been developing a multi-metal, multi-site biotic ligand model (MMMS BLM) that concurrently accounts for metal-metal competition for binding on dissolved ligands in the water and at sites of toxicity on organisms. In our initial tests, we exposed *Daphnia magna* to mixtures of Cu and Zn or mixtures of Cd and Cu in moderately hard reconstituted water containing dissolved organic carbon (DOC; added as Suwannee River fulvic acid) at 3 mg/L, and compared observed mortality to the response-additive mortality predicted from results of concurrent Cu-only and Zn-only toxicity tests or concurrent Cd-only and Cu-only toxicity tests. This research has revealed several apparent metal-metal interactions that otherwise might lead to conclusions that metals interact in non-additive ways, yet simple geochemical speciation in the BLM can explain these interactions and reconcile the apparent non-additive toxicity. For example, the toxicity of Cu-Zn mixtures always appeared to be more-than-additive or additive when based on dissolved metal concentrations, whether Cu was varied while Zn was held constant, or vice versa; whereas in the same tests, the toxicity of the Cu-Zn mixtures always appeared to be less-than-additive or additive when based on free-metal-ion concentrations. In contrast to the results for Cu-Zn mixtures, the toxicity of Cd-Cu mixtures always appeared to be less-than-additive or additive when based on dissolved metal concentrations. These preliminary results demonstrate that different metal mixtures can appear to interact differently based on dissolved-metal or free-ion concentrations, but a MMMS BLM could help reconcile those apparent inconsistencies and could be an effective tool to help water quality agencies implement more appropriate methods to regulate metal mixtures than the current default, overly conservative toxic-units approach.

**414 Effects of Maternally Transferred Methylmercury Chloride on Stress Induced Corticosterone Levels in *Nerodia sipedon* Neonates** P.W. Cusaac, Middle Tennessee State Univ, Dept of Biology; R.W. Wright, C. Henry, Middle Tennessee State Univ; F. Bailey, Middle Tennessee State Univ, Dept of Biology. Stress responses in ectothermic organisms play a crucial role in their ability to survive. Cause and response interactions of stressors are relatively well studied, however studies showing the effects of heavy metal toxicity on stress are lacking, particularly in squamate reptiles. This study was designed to examine the effects of maternally transferred methylmercury chloride on stress-induced plasma corticosterone (a hormone that normally increases in response to stress) levels in Northern Water Snake (*Nerodia sipedon*) neonates. The objective of this study was to determine if neonates from methylmercury-dosed females exhibit a diminished increase in corticosterone in response to confinement stress when compared to controls. Gravid females were dosed with 0, 10, or 10,000 ug/Kg methylmercury (3, 3, and 4 each treatment, respectively). 10 neonates from each female were used for Corticosterone testing (5 baseline and 5 stressed). Liver mercury concentrations differed significantly among the treatments (ANOVA,  $F_{(2,8)} = 9.012$ ,  $MSE = 0.04$ ,  $p = 0.016$ ). We found no significant difference of the degree of increase in corticosterone levels between dosing treatments (RM ANOVA;  $F_{(2,8)} = 1.046$ ,  $MSE = 0.018$ , H-F  $p = 0.395$ ). We therefore conclude that

methylmercury chloride has no effect on stress induced corticosterone levels in neonate *N. sipedon*.

**415 Lacertid Lizards as Bioindicators of Pesticide Exposure and Toxicity in Agricultural Areas** M. Amaral, CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, CESAM & Departament of Biology, CIBIO Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade de Aveiro, CESAM & Departamento de Biologia, Universidade de Aveiro, CESAM & Dept of Biology; M.A. Carretero, CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos; R. Bicho, Universidade de Aveiro, CESAM & Depto de Biologia; J.C. Sanchez-Hernandez, Universidad de Castilla-La Mancha, Facultad de Ciencias del Medio Ambiente; F. Guarino, Università degli Studi di Napoli Federico II, Dipartimento di Biologia Strutturale & Funzionale; A. Faustino, Universidade do Porto, Departamento de Patologia e Imunologia Molecular, ICBAS; A.M. Soares, Universidade de Aveiro, CESAM & Depto de Biologia; R.M. Mann, Univ of Aveiro, CESAM & Dept of Biology, Hydrobiology. Lizards are among the least studied groups in ecotoxicology, and despite a recent increase in the number of studies, there is still a lack of knowledge regarding their response to environmental contamination. In Europe, lacertid lizards have been identified as potential model species for reptile ecotoxicology. The main question of our project was to assess if highly abundant lacertid lizards belonging to the genus *Podarcis*, could be used as bioindicator of pesticide exposure and toxicity in agricultural areas. To achieve this end, we used a three-stage tiered approach. The first tier took the form of a field survey to document both exposure and population endpoints of lacertids occurring in areas of intensive pesticide and usage and areas of negligible pesticide usage. The second tier was a mesocosm study in which naïve lizards were exposed to pesticides in a controlled experiment. Finally, the third tier included a laboratorial approach to the effects of one of the most common insecticides used worldwide, chlorpyrifos. We assessed pesticide impact using a comprehensive set of endpoints applied at different levels of biological organization, including behavioral, physiological, biochemical and histological biomarkers. The field work confirmed the difficulty of differentiation between the effect of contaminants and other (local) factors at the population level but our results suggest a difference in the metabolic activity between animals from reference and exposed locations. Animals from exposed fields seem to be in worst body conditions and in a deficitary energy balance when compared to animals from the reference locations. The results of the mesocosm study validated the correlative data obtained in the field survey. While the laboratory approach showed that environmentally relevant dosages of chlorpyrifos can affect *P. bocagei*. According to our data, *P. bocagei* seems to be a suitable indicator of sub-lethal exposure to pesticides.

**416 Characterization and Toxicological Applications of Loggerhead Sea Turtle (*Caretta caretta*) Primary Skin Cell Cultures** S.J. Webb, G.V. Zychowski, J.M. Cole, S.L. Wiggins, Texas Tech Univ, The Institute of environmental and human health; B.M. Higgins, NOAA/NMFS. Within the past several decades, sea turtles have seen a major decline in population to the point of being listed as threatened or endangered. A large body of research has investigated several possible factors in this decline, including disruption of habitat, boat strikes, entanglement in fishing gear, and diseases such as fibropapillomatosis. However, the impact of chemical contaminants is not well-studied. Sea turtles come into contact with a variety of contaminants in the marine environment, such as polycyclic aromatic hydrocarbons, organochlorines, polychlorobiphenyls, and heavy metals. It is challenging to conduct toxicological investigations in these animals due to their threatened status. The aim of this study was to develop a method of toxicological testing using primary cell cultures developed from sea turtle skin biopsies. Here, we report on 1) the characterization of Loggerhead sea turtle (*Caretta caretta*) primary fibroblast skin cell cultures, and 2) the optimization of cytotoxicity assays such as MTT and growth curve assays. Cells were dosed with benzo[a]pyrene, a prototypical PAH and known marine contaminant, at concentrations of 10 µM, 1 µM, 100 nM, and 10 nM. Data analyses showed that RPMI 1640 medium, 30°C incubation, 10% serum concentration, and a tissue culture treated growth surface for cell adhesion were optimal growth conditions. Preliminary growth curve analyses of cells exposed to 10 µM B[a]P showed markedly lower growth, and MTT assays suggest toxicity to cells following 96-hour exposure to B[a]P at all tested concentrations. Following optimization, these toxicological assays will be useful not only in single contaminant exposures but also in examining interactions between these contaminants and other natural stressors. This

research was conducted under US Fish and Wildlife Service Endangered Species Act Section 10a(1)a Scientific Research Permit# TE\_676379\_4 and TE#676379\_5 and Florida Fish and Wildlife Conservation Commission, FWC MTP\_015 and complied with all institutional animal care guidelines.

**417 Effects of Contaminants on the Emerging Infectious Amphibian Diseases Ranavirus and Chytrid** J. Kerby, Univ of South Dakota, Biology. While both contaminant exposure and emerging infectious diseases have been implicated in recent amphibian declines, little work has been done to examine any potential interaction among these two factors. Amphibian populations can be found in areas that contain both significant levels of contamination and a high prevalence of pathogen infection. To examine some of the potential for interactions between the two, I set up three separate experiments examining the interaction of different contaminants with the pathogens Ranavirus and *Batrachochytrium dendrobatidis* (Bd). In the first experiment, I found that Tiger Salamanders (*Ambystoma tigrinum*) in the presence of chlorpyrifos (2 µg/L) and atrazine (20 µg/L) exhibited increased susceptibility to Ranavirus infection and subsequent mortality. In a subsequent experiment, the addition of a predator cue stressor with the insecticide carbaryl (500 µg/L) also significantly increased susceptibility to Ranavirus infection. In the final experiment, exposure to the antimicrobial Triclosan (100 µg/L) caused significant direct mortality to Woodhouse's Toads (*Anaxyrus woodhousii*). Exposure to lower levels of Triclosan (10 µg/L) resulted in a protective effect that actually increased survival of tadpoles also exposed to Bd. These experiments show that interactive effects can be exhibited in a laboratory setting and can both positively and negatively impact amphibian survival. Given this potential, greater attention is required in field level surveys regarding the monitoring of these two stressors and understanding the importance of their potential impacts. Additionally, further laboratory and mesocosm level experimental work should provide a greater insight to potential interactions that can occur between these two diseases and contaminant exposure.

**418 Agricultural Land Use Stresses Amphibians and Alters Immune Function** P. Falso, S. Gallipeau, Univ of California, Berkeley; T. Hayes, Univ of California – Berkeley, Dept of Integrative Biology. Amphibian populations are dramatically declining worldwide. Approximately 30% of all amphibian species are in decline and increased disease has been implicated as a major contributor. Increases in susceptibility to pathogens may result from stressful habitat conditions and subsequent disruption of immune response. Amphibian populations in agricultural regions are subject to diverse environmental stressors resulting from human habitat manipulation. The invasive American bullfrog (*Rana catesbeiana*) was used as a surrogate model for examining possible effects of agricultural contaminants and habitat disruption on sensitive native amphibian populations in the Salinas, San Joaquin, and Sacramento River systems in California. We analyzed the endocrine and immune function of bullfrogs collected at sites along a gradient of agricultural land use within these three rivers systems. Elevated plasma stress hormone levels (corticosterone) and altered white blood cell (WBC) differentials and WBC activities were observed in bullfrogs collected from agricultural sites compared to sites upstream of agriculture. In the laboratory, increased plasma corticosterone levels correlated with both altered WBC differentials and WBC activity in bullfrogs. Together these experiments give insight into the effects of stress induced by habitat alteration on endocrine and immune function in amphibians.

**419 Amphibians, Atrazine, Immunity, and Infections** J. Rohr, Univ of South Florida, Dept of Integrative Biology; T. McMahon, N. Halstead, L. Martin, T. Raffel, Univ of South Florida; S. Johnsons, Univ of Florida. Amphibians are the most threatened vertebrate taxon on the globe and many of their declines have been linked to diseases, such as chytridiomycosis, which is caused by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). Although chemical contaminants have been implicated in amphibian declines, few contaminants cause enough direct mortality at ecologically relevant concentrations to drive declines, suggesting that, if there is a link, it could be indirect and perhaps mediated by contaminant effects on infections given how important they are to amphibian losses. Using a meta-analytical approach, we reveal that exposure to atrazine, the second most commonly used herbicide and pesticide in the US, is consistently associated with immunomodulation and elevated infections in amphibians. In dose-response studies on Bd, atrazine reduced Bd growth rates suggesting that it might temporarily reduce the risk of Bd infections. However, one-week, early-life

exposure of amphibians to the expected environmental concentration of atrazine (~102 µg/L) was associated with significant immediate and long-term (7 weeks after atrazine exposure) increases in amphibian mortality due to Bd. Additionally, this increase in Bd-induced mortality was independent of when the atrazine exposure occurred during development. Our findings indicate that atrazine must be present to kill Bd, but only has to have been present in the past to cause persistent increases in the risk of Bd-induced amphibian mortality. Hence, our results support the hypothesis that the net effect of atrazine could increase the threat posed by Bd, but clearly the effects are complex and we encourage further investigations into the role of this widespread herbicide in amphibian health. Overall, this work highlights the need for more research on the role of contaminants in mediating changes in host-symbiont interactions.

**420 Effects of Contaminated Sediments, UV-Exposure, Maternal Effect and Predators on the Abnormalities Observed in Bermuda's Cane Toads (*Rhinella marina*)** J. Bacon, Bermuda Zoological Society, Research, Bermuda Zoological Society; B. Todhunter, C. Fort, H. Fort, D. Fort, Fort Environmental Laboratories. The interactive effects of contaminants, uv-exposure, maternal transfer and predators on the incidence and types of abnormalities observed were measured in newly-metamorphosed cane toads (toadlets) from four ponds contaminated with petrochemicals and metals. Abnormalities were compared in toadlets that were field-caught (FC), reared in situ predator exclusion cages (PEC), reared in laboratory microcosms exposed to media from the same four ponds (LM), and reared in laboratory microcosms exposed to three intensities of uv-light and media from two of the ponds. Comparisons were also made to predator-induced limb injuries from laboratory studies. Percent abnormal for FC, PEC and LM toadlets ranged between 14-48%, 15-59%, and 25-63% by site and year respectively. The types of limb abnormalities observed were similar in all treatments. Percent hind limb versus forelimb abnormalities, however, was statistically greater in FC toadlets, statistically less in PEC toadlets, and not statistically different in LM toadlets. In our first uv study, no abnormalities occurred in the unexposed or uv-exposed control treatments indicating that a maternal effect and uv-light alone did not induce abnormalities in this cohort. Unexposed and uv-exposed site media treatments did induce various abnormalities as expected, however. Further, concurrent exposure to uv-light and site media induced a significantly higher percentage of hind limb abnormalities (68%) than exposure to site media alone (13%). In contrast, a significant maternal effect was found in the cohort used in the second uv study; 37% of toadlets were abnormal in the unexposed control treatment. Percent abnormal in the unexposed site media treatment was significantly higher (62%), however, but the types of malformations observed were the same. Additionally, in the two uv-exposed site media treatments, both the percent abnormal (49%, 86%) and the percent of limb abnormalities involving the hind limbs (89%, 85%) were significantly higher in than the corresponding control treatments (22%, 33%), (15%, 22%). These data suggest that the types of abnormalities expressed are determined by contaminant exposure and maternal effect, but that uv-light exposure causes limb abnormalities to occur primarily in the hind limbs. Comparisons with predator-induced injuries also suggest that some cases of ectomelia and brachydactyly in field-caught specimens in Bermuda may be predator-induced.

**421 Amphibians and Nitrogen Pollution: Comparative Sensitivity and Factors Influencing Toxicity** B.K. Williams, E.E. Little, H.J. Puglis, E.L. Beahan, USGS, Columbia Environmental Research Center. Nitrogen pollution is one of the most ubiquitous stressors affecting aquatic systems worldwide. Nitrite, nitrate and ammonia pose a hazard to aquatic organisms, and have been shown to affect survival and development, induce deformities, and impair behavior in larval amphibians. In addition to causing direct injury to amphibians, nitrogen species are known to interact with other chemical and biological stressors. Improved understanding of nitrogen toxicity is necessary for elucidating interactions with other stressors, as well as for predicting the likelihood of harm to amphibian populations under field conditions. Unfortunately, published reports of nitrogen toxicity vary widely and there is a general lack of chronic toxicity data for all three nitrogen species. We conducted acute and chronic toxicity tests with nitrite, nitrate, and ammonia exposures to several amphibian species, including gray treefrogs (*Hyla versicolor*), wood frogs (*Lithobates sylvaticus*), and spotted salamanders (*Ambystoma maculatum*). Acute tests were initiated with early-stage larvae (Gosner Stage 25) and included calculation of 96-hour LC50s and EC50s



for behavioral impairment. Chronic flow-through studies were initiated with newly fertilized eggs and continued through metamorphosis (52 days for gray treefrogs and 77 days for wood frogs), with calculation of chronic LC50s and evaluation of sublethal endpoints (time to and mass at metamorphosis). These results will allow us to 1) determine the relative sensitivities of several amphibian species to nitrogen pollution, 2) compare mortality from the chronic tests with acute-to-chronic projections, and 3) derive species mean acute and chronic criterion values for these common nitrogen pollutants. In addition, the results from these studies will be discussed in the broader context of biotic and abiotic sources of variability contributing to widespread inconsistency in reported nitrogen toxicity values.

#### **422 Application of Multicriteria Decision Analysis to Support the US Army Corp's Individual and General Permitting Decisions** C. Foran,

US Army Corps of Engineers, Engineer Research and Development Center; Z. Collier, US Army Corps of Engineers, Engineer Research & Development Center; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center. USACE personnel responsible for making permitting decisions are faced with the task of evaluating multiple factors associated with proposed project alternatives. Complex tradeoffs between project alternatives containing potential environmental impacts should be assessed, but current laws and guidance document provide only general directions on such evaluations. Given the inherent complexities in environmental decision making, a structured framework is needed to guide the decision maker in a way that is transparent and repeatable. We propose applying the principles and tools of multicriteria decision analysis (MCDA) to the environmental regulatory permitting process. For individual permits, we have established a structured, multi-staged screening process that incorporates MCDA to guide regulators through the permitting process, including tools for identification of the least environmentally damaging practicable alternative (LEDPA) and for the Corps Public Interest Review. For general permits issued to states, we illustrate the application of formal decision analysis tools to enhance the current cumulative impact assessment process. Once again using an MCDA framework, we combine technical data collected in different units and qualitative expert judgment. A series of impact objectives are evaluated using criteria based on available data (i.e., GIS layers from state agencies). The relative importance of these criteria are set by assigning criteria weights and the impact scores for current and projected impacts are evaluated and compared to threshold values for the functional resource.

#### **423 Geospatial Optimization of Complex Sediment Management Decisions** M.E. Bates,

US Army Corps of Engineers, Environmental Lab, Engineer Research and Development Center; A. Tkachuk, Decision Partners; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center; M.J. Wiechmann, US Army Corps of Engineers, San Francisco District. The management and disposal of dredged materials is a complex process, with project goals of minimizing environmental risk, maximizing beneficial use, minimizing project costs and maximizing material throughput that often conflict. Through the use of optimization, GIS, and Decision Analysis, the redeveloped D2M2-J software platform is a management tool that brings transparency, flexibility, and mathematical rigor to an otherwise uncertain and subjective planning process. This approach will be especially useful in situations involving highly contaminated sediments or complex ecological interactions, and also allows users to transparently integrate stakeholder values in the decision process. The software effectively analyzes millions of combinations of dredging and placement sites to find optimal allocations of specific volumes of dredged material between sites. It optimizes management value under multiple constraints, including sediment type, dredging equipment, transportation paths, storage needs, material use, environmental concerns and other factors. The updated software will first be applied to dredged-materials management planning in the San Francisco Bay and Long Island Sound, and will be freely available for other uses. The project is currently underway with a scheduled release date in early 2012.

#### **424 Use of Conceptual Site Models in Natural Resource Damage Assessments to Promote Natural Resource Service Restoration** B. Goldsmith,

Barbara J. Goldsmith & Company, Environmental Management and Consul; T. TBD, N/A. This presentation will describe how the decision analysis tool, Conceptual Site Models (CSM), and similar models can be used to further the objectives of the natural resource damage assessment

(NRDA) process, namely to restore services provided to the public by a natural resource to its baseline through restoration, replacement or acquisition of the equivalent of the injured natural resource. Currently, there are two sets of federal regulations that provide an overall sequential framework for performing NRDA; however, the regulations are optional and do not provide an opportunity to set priorities at a specific site, make key decisions at specific junctures of an assessment, address conflicts as they arise, and ultimately resolve claims sooner. Natural resource damage (NRD) claims may be brought to restore injured, destroyed or lost natural resources (land, fish, wildlife, biota, air, water, groundwater, drinking water supplies and resources) related to oil spills or hazardous waste releases. NRD is in addition to site remediation or "clean up" requirements in those instances where natural recovery and remediation do not restore injured natural resources to baseline. Requirements for NRD claims are defined under both federal and state laws. The presentation will present how a CSM may be utilized during various stages of an NRDA in order to set priorities, resolve conflict, address key issues and restore resource and ecosystem services. The presentation will identify possible uses of CSM in the NRDA and restoration context via case example. The presentation will focus on how a CSM can be used in the NRDA and identify restoration project alternatives, including discussion on how outcomes might differ with and without the structured use of a CSM.

#### **425 Enhanced Adaptive Management: Using Decision Analytical Methods to Transparently Integrate Science, Expert Knowledge, Decisions, and Monitoring** M. Convertino,

Univ of Florida, Agricultural and Biological Engineering; M.E. Bates, US Army Corps of Engineers, Environmental Lab, Engineer Research and Development Center; J.T. Vogel II, C. Foran, US Army Corps of Engineers, Engineer Research and Development Center; J.M. Keisler, U Mass Boston, Management; A. LoSchiavo, US Army Corps of Engineers, Jacksonville District; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center. Adaptive management has been increasingly acknowledged and implemented as a means to navigate project/program success in the face of uncertain future conditions, uncertain performance of alternative management plans, and increasing resource scarcity. Many prior applications of adaptive management, however, have been criticized for falling short of their full potential. We propose to supplement existing adaptive management efforts with a comprehensive decision-analytical approach that both guides initial selection of management alternatives and allows for adjustment based on the transparent integration of monitoring information and other factors in the value of information framework. We demonstrate a hypothetical adaptive management model for a land management case study inspired by restoration needs in the Florida Everglades. The results of the model show that certain assumptions, such as the cost associated with switching between management plans, have a major impact on optimal strategy selection. The process itself demonstrates that a decision model can link science, monitoring information, and management choices to help to shape monitoring plans and move adaptive management beyond "trial and error" and provide a robust framework to guide the selection management alternatives both initially and in the future.

#### **427 NOAA's Climate Assessment and Proactive Response Initiative**

**Puget Sound Pilot** R. Neely, NOAA; M. Steinhoff, NOAA, Office of Response and Restoration; B. Shorr, A. Dvarskas, M. Baker, NOAA; A. Merten, NOAA, Coastal Response Research Center; A. Jones, D. Hudgens, N. Etre, K. Glodzik, Industrial Economics; Z. Nixon, A. Bejarano, Research Planning, Inc. The mission of NOAA's Damage Assessment, Remediation and Restoration Program (DARRP) is to protect and restore coastal and marine resources threatened or injured by oil spills and releases of hazardous substances. Hazardous waste facilities and oil infrastructure in coastal areas may be more vulnerable to releases due to climate related impacts. In response, DARRP developed the Climate Assessment and Proactive Response Initiative (CAPRI) to provide a framework and tool to evaluate potential contaminant impacts in the coastal zone related to climate change. CAPRI's flexible GIS-based framework incorporates an assessment of regionalized climate change forecasts, contaminant threats, and ecosystem and species values and sensitivities into a screening level vulnerability index. The CAPRI framework encompasses four major components: assessment of climate change impacts and related contaminant threats; development of a spatial vulnerability index; use of the web-based, open source Environmental Response Management Application (ERMA) for visualization and analysis of data layers and results; and identification of efficient prevention, response,

and restoration options. Selected sites within the Puget Sound are the initial testing ground for the CAPRI framework. This pilot incorporates Puget Sound area-specific datasets. The CAPRI framework is intended to provide a national model that can be adapted to the unique data available in a particular region or coastal area. CAPRI will enable NOAA and other local, state, regional, and federal decision makers to better prepare for and adapt to climate change by improving understanding of contaminant impacts to coastal resources.

**428 Synthetic Biology and Environmental Risks: Evaluation Using Integrated Risk Assessment, Life Cycle Assessment and Multi-Criteria Decision Analysis** A. Bockelie, MIT; E. Chu, Carnegie Mellon Univ; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center. Synthetic biology is a rapidly expanding field that has the potential to provide wide-ranging and unprecedented benefits in fields such as bioremediation to new pharmaceutical development methods. However, synthetic biology's relatively new and emergent nature creates an environment where the risks and possible effects of synthesized organisms and components are widely unknown and its current risk governance may be insufficient to properly evaluate the field's inherent risks. Additionally, the environmental behavior of synthetic biological organisms and components is unknown. If released, they may exchange genetic material across species and contaminate wild organisms. There is also the distinct possibility that the same technology in synthetic biology used to produce a vaccine could easily be used to engineer a new and deadly virus. Materials required to foster synthetic biology are becoming cheaper and more readily available to the point that virtually anyone may soon be able to create agents of biological warfare. Additionally, as synthetic biology deals with the possible creation of life, there will be ethical and social factors to consider. Governments and policy makers will be required to make informed decisions regarding tradeoffs between potential risks and benefits. Even though a traditional regulatory risk assessment framework may be applicable to synthetic biology, the lack of knowledge and significant data uncertainty will make it difficult to implement. Integrated with risk analysis and life cycle assessment, a multi-criteria decision analysis (MCDA) approach could be useful to screen an analysis of risks associated with different technologies relating to synthetic biology. The proposed framework uses LCA and RA to evaluate and assess the different risks associated with synthetic biology, including biosafety and biosecurity, at different lifecycle stages. MCDA is used to compare the risks and benefits of different technologies, products, or processes. Through the results of this model, decision makers can make informed decisions with respect to synthetic biology policy via a full understanding of the risks currently facing the field. As this framework has already been tested in an equally emergent yet uncertain field in nanotechnology, it could be useful for the emerging field of synthetic biology.

**429 Towards Sustainable Management of Contaminated Sediment – An Integrated Approach Based on Stochastic Multi-criteria Outranking Methods** M. Sparrevik, Norwegian Geotechnical Institute; M. Bates, I. Linkov, US Army Engineer Research and Development Center. Sediment contamination in a river basin or urban coastal area is often related to a complex combination of contributing sources. Contamination may have a local source, from effluent releases from present or former industrial sites, or non-local source like diffuse urban run-off or long-range pollutant transport. The sediments often act as a sink, accumulating contamination from all sources, thus making it likely to find elevated levels of contamination across large geographical areas. Large sediment volumes therefore need to be considered in remedial operations. Sustainable management of contaminated sediments will have to balance cost, environmental considerations and potentially conflicting social interests. The decision methods supporting management will therefore require use of multiple methodologies to be conclusive. The integration of these diverse decision criteria may be achieved by combining multiple analytical approaches like risk analysis (RA), life cycle analysis (LCA), multi criteria analysis (MCA) and socio-economic evaluations in an integrative framework. We propose use of stochastic multi-criteria decision analysis (MCDA) using PROMETHEE II outranking method. In the paper we have used MCDA to select the preferable magnitude of a clean material sediment capping operation for the dibenzop-dioxins and -furans (PCDD/Fs) contaminated Grenland fjord in Norway. In the analysis, positive utility for health-risk reduction and socio-economic benefit from removing the seafood bans has been evaluated against negative

utility from project cost and life-cycle environmental impact. A value-plural based weighing of criteria is compared to criteria weights mimicking a traditional cost effectiveness (CE) and cost benefit (CB) analysis. Use of this methodology may serve as a flexible framework for decision support in future cases and can be a step towards more sustainable decisions for the management of contaminated sediments and in other related areas.

**430 Impacts of Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions?** A. Boxall, Univ of York, Environment Dept; J. Staveley, Exponent; D.J. Caldwell, Johnson & Johnson; K. Choi, Seoul National Univ, School of Public Health, Seoul National Univ, Dept of Environ. Health; T. Verslycke, Gradient; S. Hickmann, German Federal Environment Agency (UBA), Pharmaceuticals Dept; K. Ostapyk, L. Innes, Health Canada; B.W. Brooks, Baylor Univ, Environmental Science and Biomedical Studies, Baylor Univ, Dept of Environmental Science; M. Rudd, Univ of York, Environment Dept. Over the past 10-15 years, a significant amount of work has been invested by the scientific, regulatory and business communities into the effects and risks of pharmaceuticals and personal care products (PPCPs) in the environment. It is now timely to review the current knowledge and to identify the key issues regarding the impacts of PPCPs in the environment so that future resources can be focused on the most important areas. This presentation describes a 20 Question Exercise that was initiated by the SETAC Pharmaceutical Advisory Group to identify the big issues that we need to address in order to better understand and manage the effects of PPCPs in the environment. In the first phase of the exercise, questions were solicited from the academic, government and business communities around the globe. A total of 403 questions were submitted which were then reviewed to remove questions outside the scope of the exercise and redundant questions, giving a final list of 101 questions. In second phase of the exercise, the 101 questions were discussed at a 2 d workshop involving academics, governmental/regulatory organisations and the business sector from N. America, Europe, Asia and Latin America and a top 20 list was developed. The top 20 questions fell into a number of broad categories, namely: a) more intelligent assessment of PPCPs; b) areas where our understanding of exposure and effects of PPCPs is less well developed; c) issues around antibiotic resistance and its control; d) risk management issues; and d) general issues, that are equally relevant to other classes of environmental contaminants. It is hoped that the conclusions from this prioritisation exercise will be used as a basis to focus future work on the risks of PPCPs in the natural environment.

**431 Response of Fathead Minnow (*Pimephales promelas*) Populations to an Estrogen Used in Human Birth Control** A. Schwindt, Colorado State Univ, Fish, Wildlife, and Conservation Biology; D. Winkelman, USGS and Colorado State Univ, Colorado Cooperative Fish and Wildlife Research Unit. Fishes in streams receiving effluent from waste water treatment plants are subjected to chemical stressors that can affect normal reproductive function. Synthetic estrogens represent one component of this effluent which can disrupt reproduction. Cellular and physiological effects are well known but little is known about potential population level consequences of reproductive disruption. We evaluated the effects of 17alpha-ethinylestradiol (EE2), the synthetic estrogen in human birth control, on fathead minnow (*Pimephales promelas*) populations. We constructed 25, 1100L aquatic mesocosms at the Colorado State Univ Foothills Fisheries Laboratory and 10 male and 10 female fish were introduced to each mesocosm. The randomized and replicated treatments consisted of EE2 concentrations ranging from 0, 2.5, 5, 10, and 20ng/L for 105 days with 5 mesocosms per treatment. Fish were allowed to behave naturally during the experiment. Adult survivorship, egg production, numbers and size of offspring, and biomarkers of estrogen exposure were collected during the experiment and used to parameterize stage-structured population models. Modeling results and physiological changes indicated that fish populations can be negatively impacted by environmentally relevant EE2 concentrations.

**432 Assessment of Estrogenic and CYP1A1-inducing Compounds in the Assabet River Basin** K. Auger, UMass Amherst, Veterinary and Animal Sciences; K. Arcaro, UMass Amherst; S. Flint, A. Field-Juma, OARS; M. Zimmerman, J. Colman, USGS. The Assabet River located in eastern Massachusetts receives treated wastewater discharges from four major municipal wastewater treatment plants: Westborough, Marlborough, Hudson and Maynard. In periods of low flow, up to 95% of the Assabet River is wastewater effluent and return flow from septic systems. Chemical analyses have

shown that municipal wastewaters can contain estrogenic and CYP1A1-inducing compounds. Estrogenic compounds such as the natural estrogen 17 $\beta$ -estradiol, the synthetic estrogen 17 $\alpha$ -ethynylestradiol, and the industrial compound nonylphenol can cause feminization in male fish through the induction of vitellogenin (VTG). CYP1A1-inducing compounds such as the polycyclic aromatic hydrocarbons and polybrominated diphenyl ethers can produce both overt toxicity and alter reproductive function through the metabolism of natural estrogens. The purpose of the present research is to assess the estrogenic and CYP1A1-inducing compounds in the Assabet River. We use both a bioassay that measures the induction of VTG and Cyp1A1 in male Japanese Medaka (*Oryzias latipes*) as well as analytical chemistry to measure the levels of selected compounds. In the summer of 2010 water samples were collected from five locations along the Assabet River and its tributaries. Male Medaka were exposed to each of the five water samples. VTG and Cyp1A1 induction were measured using quantitative RT-PCR. Concurrently collected samples were analyzed for more than 80 compounds associated with human activity including several estrogenic EDCs and CYP1A1-inducing compounds. The bioassay and chemical analysis of limited samples from season one reveal no statistically significant levels of estrogenic or CYP1A1-inducing compounds associated with stream reaches dominated by treated municipal wastewater or tributaries dominated by septic system return flow.

**433 Impact of Changes in pH on the Uptake of Pharmaceuticals and Personal Care Products into Invertebrates** M. Karlsson, Univ of York, Environment, Univ of York, Environment Dept, Central Science Laboratory, Central Science Laboratory; S. Marshall, Unilever; A. Boxall, Univ of York, Environment Dept. Following release to aquatic systems, Pharmaceuticals and Personal Care Products may associate with the sediment compartment where they may be taken up by benthic organisms. In order to establish the risks of PPCPs to benthic organisms, it is important to understand the uptake of the compounds from sediment into organisms and the effects of environmental conditions on uptake. Recent work in our laboratory has shown that the main uptake route for PPCPs into a sediment dwelling oligochaete is via the water and that uptake can be estimated based on predicted sediment pore water concentrations and bioconcentration factors from water-only studies. In this study, we extended this work to consider the effects of environmental properties (i.e., pH) on uptake of PPCPs from sediment. Uptake and depuration studies were performed for diclofenac, fluoxetine, triclosan and caffeine. The first three compounds are partly ionised at environmentally relevant pH values whereas caffeine is a neutral molecule and acted as a reference chemical in the studies. The studies were performed at pH values of 5.5, 7 and 8.5 and involved an uptake phase of 48 hours followed by a depuration phase of 48 hours. Radiolabelled chemicals were used to lower the limit of detection. The uptake and depuration rates were modelled using a two compartment model. Uptake and depuration rates were then used to calculate bioconcentration factors for the study compounds at the different pH values. Results show that bioconcentration factors can range from a log BCF of 1.0 (pH 8.5) to 5.7 (pH 5.5) for the acidic pharmaceutical diclofenac and Log BCF values for the basic pharmaceutical fluoxetine ranged 4.4 (pH 5.5) to 5.7 (pH 8.5). Changes in pH had no effect on the uptake of triclosan or caffeine. Results for diclofenac, fluoxetine and caffeine can be explained by the underlying chemistry of the study compounds. However, the observations for triclosan (an acidic compound) were not as expected. The data highlight the importance of considering the effects of pH on the uptake (and toxicity) of PPCPs in aquatic organisms. Our work is ongoing to develop modelling approaches for better predicting uptake of PPCPs from sediments under different environmental conditions.

**434 Effects of Antibiotics on the Structure and Function of Planktonic and Attached Microbial Communities from an Effluent Dominated Prairie Stream** M.J. Waiser, J.R. Lawrence, V. Tumber, Water Science and Technology Directorate, Environment Canada, Water Science and Technology Directorate; G. Swerhone, Water Sciences and Technology Directorate, Environment Canada; J. Roy, Water Science and Technology Directorate, Environment Canada. Recent worldwide surveys have established the ubiquitous presence of pharmaceuticals in surface waters receiving treated sewage effluent. Systems where such effluent dominates flow may be at the high risk for ecosystem level changes. Regina, Saskatchewan, Canada (population 190,400) treats its sewage at a modern tertiary sewage treatment facility on Wascana Creek. In winter, treated sewage effluent makes up almost 100% of stream flow. Hazard quotients calculated using existing ambient antibiotic

concentrations indicated that erythromycin (ER) and trimethoprim (TR) could present a risk to aquatic organisms. Laboratory microcosm and mesocosm experiments were subsequently, conducted to establish effects of ER, TR and clindamycin (CL) on creek planktonic and attached (biofilm) microbial communities. For planktonic communities, microcosm experiments indicated that bacterial numbers (using DAPI and epifluorescent microscope counts) and production ( $^3\text{H}$  thymidine incorporation) were significantly less than controls, especially in the ER treatments. Measures of algal biomass (Chl *a*), however, were not greatly affected. Confocal laser microscopy of biofilms grown in rotating annular reactors and exposed to varying single and mixtures antibiotic concentrations, singly and as mixtures indicated that biofilm thickness, bacterial biomass and exopolymer composition were significantly different in treated as compared to control biofilms, especially in the ER treatments. DGGE analyses on all biofilms were conducted and then data compared by principal component analysis (PCA). Results indicated that treatments TR and ER were significantly different in terms of bacterial diversity when compared to the control. Of these two, ER showed the greatest effect with its microbial community diversity grouping away from both the control and all other treatments. Results from these studies clearly indicate that antibiotics have effects on microbial community structure and function at environmentally relevant concentrations.

**435 The Fate and Effects of Triclocarban in the Aquatic Environment** C.D. Metcalfe, Trent Univ, Environmental & Resource Studies, Trent Univ, Dept of Environmental & Resource Studies; B. McIlwain, Trent Univ; T. Metcalfe, Trent Univ, ERS. Triclocarban (TCC) is an antimicrobial compound that is widely used in hard antibacterial soaps in amounts that are typically in the range of 2% by weight. The primary source of these compounds in the aquatic environment is the release of treated wastewater from municipal wastewater treatment plants (WWTPs). While there has been a considerable amount of research and regulatory focus on the ecological risks of the antimicrobial compound, triclosan, there has been relatively little scrutiny of the potential risks associated with the release of TCC. In our analysis of wastewater from several WWTPs in Ontario, Canada, up to 6.5  $\mu\text{g/L}$  of TCC were detected in untreated wastewater, but concentrations typically declined to < 1  $\mu\text{g/L}$  in treated wastewater. By deploying semi-permeable membrane devices (SPMDs) as passive samplers in surface waters in Ontario, Canada, we estimated the time weighted average concentrations of TCC in water to be as high as 0.5  $\mu\text{g/L}$ . In early life stage toxicity tests with the Japanese medaka (*Oryzias latipes*) from egg fertilization to exogenous feeding of the fry (i.e., 19 d), mortalities occurred at a nominal LOEC of 500  $\mu\text{g/L}$  and the nominal NOEC was 100  $\mu\text{g/L}$ . In partial life cycle tests with Japanese medaka exposed to nominal TCC concentrations of 10, 30 and 100  $\mu\text{g/L}$  from hatch to sexual maturity, with solution renewal every 72 h, there was a marked reduction in the production of viable eggs per female in all treatments when exposed males were mated with exposed females. Average measured TCC concentrations in the test vessels over the 72 h renewal period were about 40% of nominal. There was also evidence of masculinisation of female medaka, as indicated by the appearance of some male secondary sex characteristics. These data support an in vitro study that indicated that TCC disrupts the androgen endocrine system through an as yet unknown mechanism. Overall, these data indicate that chronic exposure of aquatic organisms to low ppb concentrations of TCC in surface waters that are impacted by discharges of municipal wastewater may cause sublethal effects, including altered development and reproductive capacity.

**436 Patterns of Removal of PPCPs from Wastewater Treatment and Their Fate in Lake Michigan: Choosing Model Chemicals for Monitoring** B.D. Blair, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences; J. Crago, Univ of Wisconsin-Milwaukee, Dept of Biology; C. Hedman, Univ of Wisconsin-Madison, State Laboratory of Hygiene; C. Magruder, Milwaukee Metropolitan Sewer District; S. Royer, Veolia Water Milwaukee; R. Treguer, Veolia Water North America; R. Klaper, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences. Pharmaceutical and Personal Care Products (PPCPs) have been recorded in wastewater effluent worldwide and the environmental impact of many of these PPCPs is unknown. Wastewater processes have not been optimized to remove influent PPCPs and increasing the predictability of which PPCP compounds need monitoring is necessary. The purpose of our experiment was to monitor the total concentrations at each stage of treatment, determine which compounds behave similarly and could be used to predict the behavior of other compounds in the wastewater treatment process, and determine the retention



and transport in Lake Michigan. PPCP and hormone concentration levels were determined for 48 compounds. Wastewater samples were collected on six different dates over a two-year period (Spring 2009 – Fall 2010) at South Shore Water Reclamation Facility (Wastewater Treatment Plant) in Milwaukee, WI. Wastewater samples were collected as raw influent, primary effluent, secondary effluent and final effluent, and the analysis was conducted using the Environment Protection Agency methods 1694 and 1698. Additionally, the fate and transport of these compounds was assessed by collecting samples at eight locations in Lake Michigan with varying proximities to the effluent discharge site. Final effluent concentrations were highest for metformin, naproxen, caffeine, sulfamethoxazole, and gemfibrozil. These compounds also had notable concentrations in Lake Michigan, up to two miles from the discharge site. Many compounds had large removal efficiencies in the wastewater treatment process. For example, the total removal of acetaminophen, caffeine, naproxen, triclocarban, and triclosan was >90%, with the majority removed by the secondary treatment process. Many compounds, such as metformin, had limited removal rates where the average raw influent and average final effluent were nearly identical. In this presentation, we will discuss the behavior of chemicals based on their properties and removal at each stage of the wastewater treatment process and discuss the fate of specific compounds in Lake Michigan.

**437 Mixtures of Pharmaceuticals in Surface Waters: Assessing Risks to Humans** K. Solomon, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Centre for Toxicology, School of Environmental Sciences. Pharmaceuticals normally have significant databases designed to evaluate possible adverse effects in humans and animals from intended and expected exposures. Even so, many individual products do not have officially determined values for acceptable daily intake (ADI) or Reference Dose (RfD). This study evaluated the potential utility of applying various approaches in a Tier 0 to Tier 1 assessment to prioritize the need for further evaluation of a mixture of residues of pharmaceuticals. The method used a Hazard Index (HI) based on the assumption of potency additivity to characterize the risk of mixtures of pharmaceuticals. This study used the Cramer classes, which have been used to define three potency bins for non-cancer endpoints in the threshold of concentration (TTC) approach. Additional evaluations of mixtures of pharmaceutical were conducted using a variety of approaches to assess the usefulness of other techniques for conducting a conservative screening. The results showed that the TTC approach based on Cramer classes was not conservative enough to use as an initial tier in the assessment of mixtures of pharmaceuticals. Use of ADIs based on the therapeutic dose divided by an uncertainty factor of 1000 to 10,000 gave HIs > 1 for maximum concentrations of pharmaceuticals reported in surface waters. When applied to reported concentrations of pharmaceuticals in drinking water, the HI was < 1, suggesting low risk. Treatment of drinking water probably resulted in reductions in exposures that further reduced risks from exposures to mixtures. Probabilistic assessment of mixtures of antibiotics measured in surface waters over time and space provided a worst case site- and day-specific HI of 0.07, suggesting negligible risk from exposures, even in the absence of any removal during treatment. The implications of these assessments will be discussed. The author acknowledges the contributions of the ILSI HESI committee on risk assessment of mixtures for their input to this case assessment which was prepared for a WHO-OECD workshop.

**438 An Evaluation of the Relationship Between DDT in Indoor Settled Dust and DDT in Outdoor Air** D. Langseth, Gradient; N. Grasso, J. Chandalia, Gradient, 29 Wachusett Drive. Due to extensive use and slow degradation rate, dichlorodiphenyltrichloroethane (DDT) and/or its metabolites dichlorodiphenyldichloroethylene (DDD) and dichlorodiphenyldichloroethane (DDE) (collectively DDT<sub>r</sub>) are nearly ubiquitous in environmental media throughout the world. Among the media in which DDT<sub>r</sub> has been detected are outdoor air and indoor settled dust. Substantial data for each of these media have been developed. The relationship between DDT<sub>r</sub> in soils and overlying air has been studied, but we are not aware that the relationship between outdoor air and indoor settled dust concentrations has been evaluated for DDT<sub>r</sub>. Such relationships have been developed for other substances, notably lead, using both empirical and mechanistic models. There is a substantial body of data on both indoor settled dust concentrations and outdoor air concentrations, but no studies of which we are aware that measured both at the same time in the same vicinity. Further, the lack of construction and activity data for individual homes in which indoor settle dust DDT<sub>r</sub> concentrations have been measured and lack of

longitudinal data for ambient air and houses in a given location severely restricts the extent to which mechanistic indoor/outdoor exchange models can be applied. Evaluation of the available data in the general framework of such mechanistic models is nevertheless instructive and suggests that a substantial portion of the DDT<sub>r</sub> found in indoor dust may be associated with DDT<sub>r</sub> in the ambient outdoor air. Other likely sources to indoor settled dust include indoor reservoirs of DDT<sub>r</sub> related to historic indoor use and drift from historic outdoor applications. Track-in from outdoor soils may in some circumstances provide historic or continuing contributions.

**439 Large Volatilization Losses of PAHs Soon After Application of Coal-Tar-Based Pavement Sealant** P. Van Metre, US Geological Survey; B. Mahler, US Geological Survey, Water Resources Discipline; M.S. Majewski, US Geological Survey; W.T. Foreman, US Geological Survey, National Water Quality Laboratory; C. Braun, J. Wilson, T. Burbank, US Geological Survey. Widespread application of coal-tar-based pavement sealants in the United States has been linked to polycyclic aromatic hydrocarbon (PAH) contamination of urban streams, lakes, soils, and indoor dust. To test whether the sealants also are a source of PAHs to air, we measured PAH concentrations in air at two heights (0.03 and 1.28 m) over a sealed pavement surface during 1 year, beginning 2 hours after sealant application in September 2009 and then at increasing time intervals (9 samples total). Air concentrations and wind speed were used to estimate volatilization fluxes. For comparison, we also measured PAHs losses from the adhered sealant, assuming losses were dominantly to the atmosphere, by measuring PAH concentrations in dried sealant and in sealant scraped from the pavement surface coincident with each air sampling. PAH concentrations initially were very high: the sum of eight PAH frequently detected in air (T-PAH<sub>8</sub>) was 297,000 ng/m<sup>3</sup> in the surface air layer (0.03-m height) 2 hours after application and T-PAH<sub>8</sub> was 26,000 mg/kg in the sealant product. After 5 days, T-PAH<sub>8</sub> in the surface air layer had decreased to 84,200 ng/m<sup>3</sup> and had decreased to 11,000 mg/kg in a sample of sealant scraped from the pavement. Concentrations in both media and the estimated flux to air decreased logarithmically during the first month after application. Concentrations in the surface air layer continued to decrease reaching a wintertime low of 301 ng/m<sup>3</sup>, before increasing the following summer to 13,200 ng/m<sup>3</sup>. Using a logarithmic function with an approximate correction for diurnal changes, the total flux of T-PAH<sub>8</sub> was estimated for the initial 14 days after application at about 2.4 g/m<sup>2</sup>. The estimated volatilization loss over this time period based on the scraping samples was about 2 times greater than the total flux estimated from the air measurements, reasonable agreement given the uncertainties in the two approaches. These results indicate that large volatilization losses occur during and soon after application of coal-tar-based pavement sealants. Combining an estimate of national annual coal-tar-based sealant use and that of PAH losses to air in the first 14 days after application from this study indicates that PAH emissions from sealants might be of the same order of magnitude as vehicle emissions of PAHs in the United States.

**440 Occurrence and Distribution Patterns of Synthetic Musk Compounds in Ambient Air from Korea** S. Oh, U. Kim, Pusan National Univ; T. Jung, Busan Metropolitan City Institute of Health and Environment; J. Oh, Pusan National Univ, Environmental Engineering. Synthetic musk compounds (SMCs) have been used to make a fragrance in various types of personal care products (e.g., deodorant, shampoo, cosmetic and others) as a fragrance substitute for natural musk. There are two types of SMCs [i.e., nitro musk (e.g., musk ketone and musk xylene) and polycyclic musk (e.g., HHCb and AHTN taking up 90% of produced SMCs)]. As the increase of widespread usage, some SMCs have been regulated and monitored as emerging persistent organic pollutants in European Union due to their persistency, bio-accumulation and the possibility of toxicity effect on human and environment. In various media, SMCs have been detected on the order of a few ng/m<sup>3</sup> down to pg/m<sup>3</sup> levels in environment and especially, some of researchers have been reported at the levels of 1 to 5 ng/m<sup>3</sup> in ambient air (HHCb and AHTN in gas phase). Moreover, even remote regionlike the lake of the Alps, the trace levels of SMCs contamination has been detected. However, there are no proper data about SMCs occurrence in ambient air in Korea. Therefore, the objective of this paper is to investigate the occurrence and the fate of SMCs in Korean ambient air. High volume samplers (SIBA-TA, HV-1000) were used to collect gas and particle phase of SMCs with PUF (polyurethane foam) and GFF (glass fiber filter), respectively, from five different sampling sites in Busan, Korea. GC/EI-MS was used to analyze SMCs in air samples with selected ion monitoring mode. The target SMCs

are six polycyclic musk compounds (HHCB, AHTN, AHDI, AHMI, ATII and DPMI) and nitro musk compounds (musk xylene). 3 PMCs (HHCB, AHTN, DPMI) and musk xylene were detected in all samples. HHCB concentrations ranged from 3.43 to 801.58 pg/m<sup>3</sup> and AHTN concentration was from 3.38 to 195.38 pg/m<sup>3</sup>. MX and DPMI were observed from 8.61 to 277.13 pg/m<sup>3</sup>; and 10.51 to 27.51 pg/m<sup>3</sup>, respectively. Other compounds (ATII, ADBI and AHDI) were detected below 10% of the total SMCs load. The detailed discussion about SMCs' distribution pattern between gas and particulate phase and seasonal variation of SMCs concentration will be presented in the conference.

**441 Deposition and Accumulation of Airborne Organic Contaminants in Yosemite National Park, California** D. Alvarez, US Geological Survey, USGS Columbia Environmental Research Center; M.A. Mast, USGS Colorado Water Science Center; S. Zaugg, US Geological Survey, Methods Research and Development Group, National Water Quality Laboratory; W. Cranor, USGS Columbia Environmental Research Center; H. Forrester, National Park Service, Yosemite National Park. Deposition and accumulation of airborne organic contaminants in Yosemite National Park were examined by sampling wet deposition (snow and rain), lichen, and lake sediment at different elevations in the park during 2008-2009. In 2009, the semipermeable membrane device (SPMD) passive samplers were deployed in ten high-elevation lakes to estimate surface-water concentrations. Frequently detected compounds included the current-use pesticides endosulfan, dacthal, and chlorpyrifos and the legacy pesticides DDD/DDE, chlordane, HCB, dieldrin, and PCBs. Snowpack concentrations were fairly uniform and showed little variation with elevation. Temporal variation in rain concentrations of endosulfan appeared to coincide with application rates in the San Joaquin Valley. More than 70% of annual pesticide inputs from wet deposition occurred during winter largely because most precipitation at high elevations falls as snow. Lichen concentrations showed positive gradients with elevation for some compounds indicating cold condensation may be an important control on pesticide accumulation particularly endosulfan and chlordane. The opposite pattern in lichens was found for chlorpyrifos likely reflecting high application rates of this pesticide in nearby agricultural areas. Sediment concentrations were inversely correlated with elevation partly due to organic carbon content of sediments but also perhaps greater mobility of organic contaminants at lower elevations. Surface-water concentrations inferred from passive samplers were at sub part per trillion concentrations indicating minimal exposure to aquatic organisms from the water column. Sediment concentrations generally were low except for DDD in Tenaya Lake, which exceeded sediment guidelines for protection of benthic organisms.

**442 Trends of Current Use Pesticides (CUPs) in Canadian Arctic Air and Water: 1999-2010** A. Gawor, Univ of Toronto, Dept of Chemistry; F. Wania, Univ of Toronto Scarborough, Dept of Physical & Enviro. Science, Univ of Toronto at Scarborough, Dept of Physical & Enviro. Science; L.M. Jantunen, T.F. Bidleman, Environment Canada; F. Wong, Univ of Toronto, Chemistry; G. Stern, Freshwater Institute, Dept of Fisheries and Oceans; H. Kylin, Swedish Univ of Agricultural Sciences, Aquatic Sciences and Assessment; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch. Since the ban on organochlorine pesticides (OCPs), the use of other forms of insecticides, fungicides and herbicides, otherwise known as current use pesticides (CUPs), have increased. CUPs are generally less persistent than the older style OCPs, however, some can still undergo atmospheric transport. Transport of pesticides from agricultural fields to non-target areas is of great interest because their occurrence in remote regions is recognized as evidence for long-range transport under international protocols, which were implemented to control persistent, bioaccumulative, and toxic substances. Some CUPs ultimately make their way to the Canadian Arctic, where they were detected in air, water, ice, and biotic species. Due to colder conditions, they persist in the environment for longer periods of time. CUPs that were detected in Arctic air and water include: dacthal, chlorpyrifos, endosulfan-I, trifluralin, chlorothalonil and pentachlorothioanisole. All of these CUPs are still in use in Canada, although dacthal has very limited usage. Temporal and spatial trends for these contaminants are essential to understand the effect that CUPs will have on the environment; especially since the diminishing Arctic sea-ice due to warming temperatures may change the mobility of the chemicals in the environment. Air and water samples were collected as part of Tundra North-west-99, the International Polar Year 2007-2008 and ArcticNet-2010 to

determine the occurrence and levels of CUPs over time. Results have shown that for some CUPs, specifically endosulfan and chlorothalonil, concentrations have increased in the Arctic water between 1999-2010, whereas CUPs like dacthal, concentrations have remained relatively constant.

**443 Persistent Organic Pollutants in the Atlantic and Southern Oceans and Oceanic Atmosphere** J. Luck, R.M. Dickhut, Virginia Institute of Marine Science; R. Falconer, Chatham Univ, Chatham College, Dept of Chemistry; M. Cochran, Virginia Institute of Marine Science; H. Kylin, Swedish Univ of Agricultural Sciences, Aquatic Sciences and Assessment. Persistent organic pollutants (POPs) are toxic chemicals that bioaccumulate and biomagnify in organisms, and many POPs undergo atmospheric transport to remote areas. The Atlantic Ocean and the Western Antarctic Ice sheet may be sinks for currently used agricultural and industrial organohalogen compounds, while these regions may be sources for POPs that have been severely restricted and banned. Air and seawater samples were collected aboard the Swedish Icebreaker Oden as it traveled from Goteberg, Sweden to McMurdo Station, Antarctica during Oct. – Dec. 2007. These samples were analyzed for POPs to examine the current global distribution of POPs in the marine waters and atmosphere. Both air and surface seawater samples consistently contained  $\alpha$ -hexachlorocyclohexane ( $\alpha$ -HCH), g-HCH, and hexachlorobenzene (HCB), while air samples additionally contained several polychlorinated biphenyls (PCBs) and a-Endosulfan. Sample concentrations for most compounds in air were significantly higher in the northern hemisphere ( $p < 0.01$ ), with the exception of HCB, which had high concentrations in the northern and southern latitudes and low concentrations near the equator. Air and seawater concentrations of a- and g-HCH were compared to determine the net flux of these pollutants between the air and seawater, and with the exception of  $\alpha$ -HCH in the northern most waters of the Atlantic, Atlantic and Southern Ocean waters were found to be a sink for both a- and g-HCH.

**444 Pesticides in Rain in the Mississippi River Valley: A Comparison Between 1995 and 2007** M.S. Majewski, S.J. Kalkhoff, R.H. Coupe, P.D. Capel, US Geological Survey; W.T. Foreman, US Geological Survey, National Water Quality Laboratory. Weekly composite rainfall samples were collected during the 1995 and 2007 growing seasons at two agricultural locations in both Iowa and Mississippi, and analyzed for 47 pesticides and transformation products (TPs) in 1995, and 76 in 2007. Thirty two analytes were detected at least once in one or both years; 11 analytes were detected both years. Herbicides were the predominant type of pesticide detected in both years and states. In Iowa, 15 herbicides, 7 insecticides, and 2 TPs were detected in 1995 with total flux of 445  $\mu\text{g}/\text{m}^2/\text{season}$ . Nine pesticides detected in 50% or more of the samples accounted for 94% of the deposited mass. In 2007, 8 herbicides, 3 insecticides, 2 fungicides, and 4 TPs were detected with a total flux of 186  $\mu\text{g}/\text{m}^2/\text{season}$ . Six pesticides detected in 50% or more of the samples accounted for 86% of the flux. Acetochlor, alachlor, atrazine, CIAT (an atrazine TP), DCPA, and chlorpyrifos were among the top 10 most frequently detected compounds in both years. In Mississippi, 16 herbicides, 9 insecticides, and 1 TP were detected in 1995 with a total flux of 1981  $\mu\text{g}/\text{m}^2/\text{season}$ , but methyl parathion accounted for 90% of this flux. Nine pesticides that were detected in 50% or more of the samples accounted for 98% (83% not including methyl parathion) of the deposited mass. In 2007, 11 herbicides, 6 insecticides, 2 fungicides, and 6 TPs were detected with a total flux of 436  $\mu\text{g}/\text{m}^2/\text{season}$ . Five pesticides detected in 50% or more of the samples accounted for 54% of the flux. Atrazine, metolachlor, and propanil were among the top 5 compounds detected in rain in both years in Mississippi. The amount, timing, and frequency of rainfall play a critical role in when and which pesticides are detected, their concentrations, and their seasonal flux. During the spring when most herbicides are applied, rain events in Iowa were more frequent in 1995 than in 2007. This was reflected by higher detection frequencies, mean concentrations, and total flux in 1995. In Mississippi, rain events occurred more uniformly throughout the growing season in both years, yet the total pesticide flux increased from 1995 to 2007 while the number of compounds detected at greater than 50% decreased by about a half. Much of the increased 2007 deposition amount is attributed to the considerable increase in use of atrazine and glyphosate and the number of compounds detected below 50% frequency that contributed significantly to the total flux, 7 in 2007 versus 2 in 1995.

#### 445 Comparison of the Atmospheric Occurrence and Deposition of Glyphosate and Conventional Herbicides in Two Agricultural Areas of the United States

P. Capel, US Geological Survey; F. Chang, Clarkson Univ; M. Simcik, Univ of Minnesota; M. Majewski, W. Foreman, S. Kalkhoff, R. Coupe, US Geological Survey. The use of herbicides to combat weeds is an important practice in modern agriculture. During the 1990s, crops which were genetically-modified (GM) to tolerate the effects of a specific herbicide, most commonly glyphosate, were introduced and widely accepted. In 2007, glyphosate-tolerant GM-soybeans, cotton and corn comprised 95, 80 and 59 percent of these crops, respectively. The widespread adoption of herbicide-tolerant GM crops has caused a major shift away from the use of conventional pre-emergent herbicides (triazines, acetanilides, etc.) towards glyphosate. During the 2007 growing season, the air and rain at agricultural sites in Iowa and Mississippi were monitored for 23 herbicides, including glyphosate. Glyphosate, a relatively non-volatile compound, enters the atmosphere primarily through application spray drift and wind erosion. In Iowa, the median glyphosate concentration in rain (0.20 mg/L) was 10 to 100 times greater than the median concentrations of metolachlor, acetochlor and atrazine, although their detection frequencies were similar (65, 76, 71 and 59% percent for glyphosate, metolachlor, acetochlor and atrazine, respectively). The results were similar at the site in Mississippi where median glyphosate concentration in rain was 0.25 mg/L, about 10 to 100 times greater than the median concentrations of atrazine, metolachlor, and propanil. In air, glyphosate was detected less frequently (61 and 86% in Iowa and Mississippi) than the most common conventional herbicides such as atrazine, trifluralin and metolachlor, but at similar median concentrations. Wet deposition of glyphosate during the growing season was calculated to be 50 and 130  $\mu\text{g}/\text{m}^2$  compared to 90 and 104  $\mu\text{g}/\text{m}^2$  for the sum of the other 22 targeted conventional herbicides in Iowa and Mississippi, respectively. Thus, although it is non-volatile, the atmospheric occurrence, concentration, and depositional fluxes of glyphosate are similar to or greater than the more volatile conventional herbicides due to the sheer magnitude of its use.

#### 446 Evolving Role of Passive Samplers in Whole Sediment Toxicity Identification Evaluations

R.M. Burgess, USEPA, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, USEPA, USEPA; M.M. Perron, Brown Univ; K.T. Ho, M.G. Cantwell, USEPA, Atlantic Ecology Division. In Phase I of whole sediment TIEs, causes of toxicity to freshwater and marine organisms are characterized into broad toxicant classes including ammonia, metals and organic chemicals. In Phase II of the TIE, the specific toxicants causing observed toxicity are identified. For ammonia and metals, this process is often not complex because of the limited number of metals likely to cause toxic effects and because ammonia toxicity is well understood. In contrast, identifying the specific organic chemicals causing toxicity is very challenging because of the thousands of chemicals that may be present and contributing to adverse effects. In effects directed assay (EDA) as developed in Europe, a major focus is on the identification of toxic organic chemicals using sophisticated extraction, fractionation and analytical methods often with cellular endpoints in artificial media. In the TIE approach developed in North America, the emphasis has been on exposing whole organisms in systems emulating environmental conditions including contaminant bioavailability. A consequence of the TIE strategy has been limited development of sophisticated identification methods for organic chemicals. Given that most Phase I whole sediment TIEs indicate organic chemicals are the cause of toxicity, more sophisticated Phase II identification methods are needed. A major challenge is the development of exposure techniques for whole organisms that reduce the mixture of organic chemicals potentially causing toxicity into simpler components that can be analyzed for identification. In this presentation, the evolving role of passive samplers in whole sediment TIEs will be discussed as a means for generating "simplified" sediment-based exposures that allow for identification. Use of whole sediment extraction schemes that better reflect contaminant bioaccessibility and bioavailability will be described. Additionally, the application of semi-permeable membrane devices and reverse polyethylene devices as dosing systems will be reviewed. Toxicological and chemical information will be provided evaluating the different approaches. This presentation will provide information useful for the development of identification processes for organic chemicals causing toxicity to whole organisms in freshwater and marine environments.

#### 447 Determining the Causes of Sediment Toxicity in California

B. Philips, Univ of California – Davis, Environmental Toxicology; B. Anderson,

Univ of California – Davis, Dept of Environmental Toxicology; J. Voorhees, University of California – Davis, MPSTL – Environmental Toxicology; K. Siegler, Univ of California Davis; R. Tjeerdema, Univ of California – Davis, Environmental Toxicology. Sediment toxicity is pervasive in California watersheds. A recent summary of statewide data showed 47% of ambient samples were toxic to amphipods. This has regulatory implications because many California water bodies are listed as degraded due to ambient toxicity. It is necessary to determine the cause of toxicity so that contaminant sources can be identified and controlled. Research at UC Davis combines toxicity identification evaluations (TIEs) with other procedures designed to separate the effects of contaminant and non-contaminant factors, and to link laboratory effects with ecosystem impacts. Recent research has emphasized current-use pesticides such as organophosphates and synthetic pyrethroids. As part of recent monitoring, pyrethroids were detected in half of sediments collected at the base of watersheds, in toxic sediments collected in urban creeks, and in near-coastal marine sediments. Some observed toxicity was attributed to pyrethroids using several lines of evidence. Median lethal concentrations (LC50s) are used to determine if measured contaminant concentrations are greater than toxicity thresholds. Carbon-corrected LC50s allow for more accurate prediction of the bioavailable fraction of contaminants. Dose-response information developed for standard laboratory and resident species provide an important diagnostic tool. Because of their high toxicity and low solubility, accurate measurement of pyrethroids is problematic, and in many cases TIEs are relied upon to provide evidence for pyrethroid toxicity. Specific TIE methods for pyrethroids include addition of enzymes to break down pyrethroids, addition of piperonyl butoxide to inhibit pyrethroid metabolism, and manipulation of testing temperatures. When combined with solid-phase extraction techniques, and chemical analysis, these methods provide evidence for pyrethroid toxicity. Macroinvertebrate bioassessments are the primary tool to assess ecosystem impacts in California watersheds. Measures of contaminant and non-contaminant factors, habitat, and toxicity testing provide data linking stressors with impacts. Primary stressors identified through correlation analyses are further investigated using laboratory experiments, including dose-response experiments with key resident fauna. Data from this research has indicated the relative contributions of current-use pesticides to aquatic habitat degradation in California.

#### 448 Identification of Compounds in Heavy Oil that are Toxic to Fish Embryos Using Effect-driven Chemical Fractionation and Chronic Toxicity Tests

J. Adams; J. Bornstein, Queen's Univ, Chemistry, Queen's Univ, Chemistry Dept; P.V. Hodson, Queen's Univ, School of Environmental Studies. Heavy fuel oil (HFO) is the refined product of crude oil distillation that is used in ships and large heating plants. When this thick, viscous oil is spilt in aquatic environments it does not behave like lighter oils that float; HFO sinks within the water column and accumulates in sediments, affecting aquatic organisms that are not typically exposed to floating oils. Effect-driven chemical fractionation (EDCF) of crude oil has identified the 3-5 ringed alkylated polycyclic aromatic hydrocarbons (PAHs) as the major toxic fraction. HFO is comprised of a higher concentration of 3-4 ringed alkyl PAH and an abundance of 5-6 ringed PAH, and is predicted to be more toxic to fish. There has been little chemical characterization and identification of the compounds within HFO responsible for toxicity. This research is the first ever EDCF of HFO based on embryo toxicity tests conducted to identify the specific classes of alkyl PAH associated with toxicity, determine whether these components are sufficiently bioavailable to cause toxicity and establish the toxicity of HFO relative to medium and light oils.

#### 449 Toxicological and Chemical Profiling of Contaminated Surface Waters by Holistic Passive Sampling Approach

C. Liscio, Univ of Sussex, Biology and Environmental Science; E. Hill, Univ of Sussex. Environmental monitoring represents an on-going challenge and constant improvements in analytical methods have further increased awareness of the presence of contaminants at trace levels which can exert harmful effects on the environment. Amongst these contaminants, Endocrine Disrupting Compounds (EDCs) are being included in European and international conventions for the protection of aquatic environment, especially of surface waters. Identifying the causative agents of endocrine disruption is of paramount importance given that EDCs potentially have very wide reaching deleterious impacts to wildlife and possibly humans too. Thus far, endocrine disruption has been mostly focussed on estrogenic activity, but recently the presence of antiandrogenic compounds in contaminated aquatic environments has



raised scientific concern. Structures of chemicals containing androgen receptor antagonist properties can be extremely diverse and it is important to use methods to identify antiandrogens which do not make any assumptions as to the nature of the chemicals involved. A bioassay-directed fractionation is the best approach to isolate antiandrogenic chemicals in complex environmental extracts and to allow further identification of the unknown active structures. However, water sampling can result particularly problematic for EDCs, since they are present at ultra-trace levels and encompass a variety of chemical classes differing significantly in physical-chemical properties. A holistic passive sampling approach can be an efficient alternative to overcome these limitations: the use of a combination of different passive samplers may allow sampling of a wide range of compounds with a significant preconcentration from surface waters, increasing the likelihood of successful identification of the chemical structures. In this study, 4 different passive samplers were used to detect antiandrogenic activity present in different contaminated surface waters. Samples were fractionated and profiles derived from the 4 passive samplers were compared and characterized. The nature of key antiandrogenic contaminants was investigated by mass spectrometry techniques. In addition, a general chemical profile of contaminants present in the waters was obtained using UPLC-TOF MS analysis of the unfractionated passive sampler extracts. Chemometrics tools were then used to model information-rich chemical data and to identify key classes of chemical contaminants associated with the different samplers.

**450 Predicting Bioavailability of Polycyclic Aromatic Hydrocarbons to Soft-shelled Clams Using In Situ Deployment of Polyethylene Passive Samplers** L.A. Fernandez, US Environmental Protection Agency, Office of Research and Development; P.M. Gschwend, Massachusetts Institute of Technology, Dept of Civil and Environmental Engineering. Freely-dissolved porewater PAH concentrations and chemical activities have been suggested as measures of the bioavailability of PAHs in contaminated sediment beds. However, the difficulties of measuring porewater concentrations and reaching equilibrium during laboratory exposure studies have made it challenging to correlate chemical activity with bioavailability. Passive sampling methods make it possible to measure porewater concentrations and chemical activities without disturbing sediment beds or returning bulk sediment samples to the laboratory. Sampling native organisms also ensures tissues are at a natural equilibration with sampled sediments. In this work, polyethylene (PE) passive samplers, containing performance reference compounds (PRCs) (d10-phenanthrene, d10-pyrene, and d12-chrysene), were deployed in sediment beds near Boston, MA (USA) for a one-week period. Clams (*Mya arenaria*) and sediments were then collected from the same sediments (directly adjacent to samplers). Concentrations and chemical activities of three PAHs that matched the PRCs (phenanthrene, pyrene, and chrysene) were measured in the porewaters (using PE-PRC method), in clam tissues, and in the bulk sediment. Chemical activities in clams were compared to (a) those measured in porewaters using PE samplers and (b) those calculated using sediment measurements, and equilibrium partitioning (EqP) models that includes sorption to organic carbon and black carbon fractions. While no statistically significant correlations between chemical activities in clams and sediment porewaters calculated using EqP models were found, correlations were seen when using PE to derive porewater chemical activities for both pyrene and chrysene ( $r^2$  of 0.29 and 0.93, respectively). Ratios of porewater and organism chemical activities indicate that the clams were nearly equilibrated (within a factor of 2) with sediment porewaters for phenanthrene and chrysene, while pyrene porewater chemical activities were higher than those in organisms by about a factor of 5. EqP models overestimated porewater concentrations for the three chemicals by one to three orders of magnitude at all but one site. These data indicate the use of chemical activities based on passive sampler measurements are valuable predictors of PAH bioavailability.

**451 Development of a Gene Expression Approach for Sediment Toxicity Identification** S.M. Bay, Southern California Coastal Water Research Project, Toxicology Dept; C. Vulpe, Univ of California – Berkeley, Nutritional Science and Toxicology. Identification of the cause of sediment toxicity is needed in order to design effective management actions to improve sediment quality. Sediment TIEs based on traditional methods are often limited in their usefulness when multiple potential stressors are present, the magnitude of toxicity is low, or when the chemical characteristics of the toxicant are not very distinctive. A potential new method for stressor identification in sediment toxicity tests is under development that is based on measuring changes in gene expression. A custom microarray containing over 10,000

probes for gene expression was developed from the expressed genome in the marine amphipod *Eohaustorius estuarius*, a species widely used for sediment toxicity assessment. Differential gene expression in *E. estuarius* was measured following exposure to a variety of toxicants, including chlorinated pesticides, metals, current use pesticides, and PAHs. Distinctive patterns of gene expression were identified for most contaminants, suggesting that development of a diagnostic tool for stressor identification is feasible. Several sets of diagnostic genes and associated classification models were developed using multivariate statistical methods. Results from the evaluation of the classification models using independent validation samples from lab and field studies are described. The influence of population variability, exposure matrix, and dose on gene expression patterns and classification ability were also investigated. Use of gene expression is a promising tool for sediment TIE.

**452 Integration of Gene Expression Biomarkers and Whole Sediment Toxicity Identification Evaluations** A. Biales, USEPA, National Exposure Research Laboratory, USEPA, ORD/NERL, USEPA; M. Kostich, USEPA, NERL, National Exposure Research Laboratory, USEPA; R.M. Burgess, USEPA, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, USEPA, USEPA; M. Pelletier, National Health and Environmental Effects Research Laboratory, USEPA, Dept: ORD NHEERL AED; L. Portis, National Health and Environmental Effects Research Laboratory, USEPA; M. Perron, Brown Univ, School of Engineering; K. Ho, National Health and Environmental Effects Research Laboratory, USEPA. Toxicity Identification and Evaluations (TIEs) use physical/chemical manipulation of a sample to isolate or change the potency of different groups of chemicals potentially toxic in a sample. Organisms are then exposed to these fractions pre- and post- manipulation to determine if the toxicity has changed. TIEs often focus on mortality to detect toxicity; however, this endpoint gives no definitive information as to the identity of the active toxicant(s). It has been suggested that gene expression profiles may be useful in identifying toxic substances from unknown samples. This is accomplished through the development of expression profiles, consisting of a number of genes, which respond in a specific manner to a given toxicant. Generally these expression profiles are established through the use of global transcriptional platforms, such as microarrays. For the current project, microarrays were constructed for the amphipod *Ampelisca abdita*, a model organism employed in sediment TIEs, which previously lacked any characterization on the molecular level. Exposures to eight model toxicants and mixtures were conducted at 10% of the LC50. Gene expression signatures were generated for individual exposures to specific toxicants or groups of toxicants. Using Elizabeth River, VA sediments (ER), TIEs were conducted at two sediment dilutions, one toxic (40:60; ER:control sediment; 86.7% mortality) and one non-toxic (10:90; ER:control sediment; 0% mortality). A gene expression classifier was generated that would discriminate between ER and control sediment samples. Gene expression profiles of model toxicants, ER exposed organisms and control were clustered, with the idea that the model toxicant responsible for the observed mortality in the 40% ER sample would cluster more closely to the unmanipulated ER sediment relative to the other model toxicants. TIE results of the 40% ER samples, indicated a large decrease in mortality rates with coconut charcoal addition and a moderate decrease with cation exchange resin addition. These results were compared with results obtained using gene expression as an endpoint. Comparisons were also made between results obtained using TIE and gene expression in the 10% ER sediment, where no significant results using the TIE process were obtained. This work demonstrates the utility of incorporating gene expression endpoints to confirm and accentuate whole sediment TIEs.

**453 EDA of Unknown Toxicants in the Environment – Strategies to Identify Compounds Based on Chromatography, Mass Spectrometry and Calculated Properties** W. Brack, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis, UFZ Centre for Environm. Research, Dept of Chemical Ecotoxicology; C. Hug, Helmholtz Centre for Environmental Research-UFZ, Effect-Directed Analysis; M. Krauss, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis, Helmholtz Centre for Environmental Research, Effect-Directed Analysis; M. Markus, German Aerospace Center DLR, Remote Sensing Technology Institute; N. Steffen, Leibniz Institute of Plant Biochemistry, Dept for Stress and Development Biology; T. Schulze, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis; S. Emma, Helmholtz Centre for Environmental Research UFZ, Effekt-Directed Analysis, EAWAG – Swiss

Federal Institute of Aquatic Science and Technology; U. Nadin, P.C. Vonderohe, Helmholtz Centre for Environmental Research UFZ. While typical environmental mixtures contain thousands to ten thousands of compounds, the risk to ecosystems and human health is typically estimated on the basis of a few known target compounds, which are often unable to explain measurable effects. Effect-directed analysis (EDA) is a valuable approach to identify causative toxicants but is complicated by the presence of many unknowns detected during screening of toxic fractions but present at concentrations and purity insufficient for structure elucidation e.g., by NMR spectroscopy. To meet this challenge, we present a systematic strategy for compound identification based on chromatography, mass spectrometry and calculated properties. Firstly, database searching based on GC-MS and LC-MS is performed, considering (exact) mass, fragmentation and retention information. Alternatively, structure generation combined with substructural classifiers can be used to generate candidate structures. Secondly, a systematic reduction of the number of candidates is achieved by comparing predicted and measured retention behaviour in GC and LC, using a steric energy calculation, predicting mass spectral fragmentation patterns and finally application of QSARs and structural alerts for effect prediction. This strategy is demonstrated using examples applying this approach in EDA studies. To enhance the sharing and interpretation of especially LC-MS(n) data, we also introduce a non-commercial common database to achieve optimal synergism of chemical screening and EDA worldwide.

**454 Inhibition of Thyroid Hormone Sulfotransferase Activity by Brominated Flame Retardants and Halogenated Phenolics** C. Butt, H.M. Stapleton, Duke Univ, Nicholas School of the Environment. The maintenance of thyroid hormone (TH) homeostasis is complex, but critical for normal physical and mental development. The biologically active TH is 3,5,5'-triiodothyronine (T3), which is formed through deiodination of the T4 phenolic ring via deiodinase enzymes (DI). Deiodination can also occur on the tyrosyl ring resulting in the formation of 3,3',5'-triiodothyronine (rT3). Both T3 and rT3 are further deiodinated to yield 3,3'-diiodothyronine (3,3'-T2). The rT3 and 3,3'-T2 are thought to be biologically inactive. In addition to deiodination, THs undergo phase II metabolism via conjugation of the hydroxyl group with glucuronic acid or sulfate. Interestingly, the sulfation of some THs increases their deiodination efficiency as compared to non-sulfated analogue. In this study, we investigated the potential of halogenated phenolic compounds to inhibit TH sulfation using *in vitro* assays. We used 3,3'-T2 as the substrate since it has been shown that this compound is the most efficient TH compound for sulfation and our model system was human liver cytosol. THs and the 3,3'-T2 sulfated conjugate were analyzed by LC-MS/MS, adapted from previous methods developed in our lab. We tested several brominated flame retardants (tetrabromobisphenol A, 2,4,6-tribromophenol), PBDE metabolites (hydroxylated BDEs) and triclosan as potential TH sulfation competitors. Further, we explored structure-activity relationships by investigating TH sulfation inhibition by fluorinated, chlorinated and iodinated analogues. All compounds tested showed the ability to inhibit 3,3'-T2 sulfation in a dose-response manner. In general, the OH-BDEs were the most potent inhibitors (IC<sub>50</sub> values: 50-340 nM) followed by TBBPA and triclosan. Differences were observed between the various OH-BDE congeners and structure-activity relationships were developed. For example, the 3-OH BDE 47 was a more potent inhibitor than the 5-OH BDE 47. The present study demonstrated that several brominated flame retardants and the hydroxylated metabolites of PBDEs (as well as the anti-microbial, triclosan) can inhibit TH sulfation. This potentially may impact TH metabolism and thus may influence circulating TH levels in the body. This study contributes to the growing body of literature demonstrating that halogenated phenolics may disrupt TH homeostasis.

**455 Determination and Characterization of Hydroxylated PCB Metabolites (OH-PCBs) in 1959-1967 Archived Serum from California Women: The Follow-up Study** D. Tarrant, California Environmental Protection Agency, Dept of Toxic Substances Control; A. Rhee, California Environmental Protection Agency, Dept of Toxic Substances Control, Public Health Institute; S. Harwani, J. Park, M. Petreas, California Environmental Protection Agency, Dept of Toxic Substances Control; B. Cohn, P. Cirillo, Center for Research on Women's and Children's Health; P. Factor-Litvak, Columbia Univ, Dept of Epidemiology. The hydroxylated metabolites of PCBs (OH-PCBs) are formed and retained by the body, and may have adverse effects on the thyroid system. These metabolites compete and replace thyroxine (T4) thyroid hormone in binding to the transport protein

transthyretin (TTR). Replacement of thyroxine with hydroxylated PCBs can result in endocrine related problems associated with hypothyroidism, deficits in neurodevelopment and immune functions, and impaired reproductive system in offspring. The initial pilot study that characterized and quantified the levels of specific PCBs and OH-PCBs metabolites from 30 archived third trimester or postpartum serum specimens from California mothers during 1959-1967 was successful in assessing the feasibility of measuring the OH-PCB metabolites in 1 mL of archived sera. The OH-PCB concentrations were measured at levels that are likely to be toxicologically significant, and are comparable to other studies that showed toxicological effects of these metabolites. The objective of our current research is to quantify and characterize the OH-PCB metabolites and their parent PCBs in the larger cohort (N=510) of archived maternal sera from 1959-1967. The PCB exposure levels are expected to be higher than current levels, since these maternal samples were acquired in a time period prior to regulatory action that banned the production of PCBs. Our results indicate that the sera samples contain 4-OH-CB187 and 4-OH-CB 107 as the primary and secondary metabolites respectively. This is the first large scale study to assess OH-PCB levels in a US population. The ultimate goal for this study population is to explore associations between OH-PCBs metabolites, PCBs, and thyroid effects. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Dept of Toxic Substances Control, California Environmental Protection Agency.

**456 Polybrominated Diphenyl Ethers (PBDEs) and Their Hydroxylated Metabolites in Paired Maternal and Cord Serums Collected in Cincinnati** A. Chen, Univ of Cincinnati; J. Park, California Environmental Protection Agency, Dept of Toxic Substances Control, California Environmental Protection Agency, Research Scientist; A. Rhee, M. Petreas, California Environmental Protection Agency, 700 Heinz Ave.; E.A. DeFranco, K.N. Dietrich, S. Ho, Univ of Cincinnati. Polybrominated diphenyl ethers (PBDEs) were widely used as flame retardants in the past three decades. These compounds are lipophilic and easily cross the placenta from mother to fetus. It is not clear whether hydroxylated PBDEs (OH-PBDEs), metabolites of PBDEs with more structural similarity to thyroid hormones but more hydrophilic, differ in maternal and cord blood samples. We analyzed both PBDEs (BDE-28, -47, -99, -100, -153, -154, -209) and OH-PBDEs (6-OH-BDE-47, 5-OH-BDE-47, 4'-OH-BDE-49, 5'-OH-BDE-99) in a pilot of first 10 pairs of maternal and cord serum samples collected in Cincinnati, OH in 2011. We used standard liquid-liquid serum extraction and phase separation techniques. Analysis of PBDE was performed by gas chromatography/high resolution mass spectrometry (GC-HRMS, Thermo-Finnigan) coupled with isotope dilution. OH-PBDEs were determined as methyl derivatives (MeO-PBDEs) by using Varian 1200 GC/MS with negative chemical ionization mode. The median concentration of  $\Sigma(7)$ PBDEs was 35 ng/g lipid (24-104) in maternal serum and 43 ng/g lipid (range 25-117 ng/g lipid) in cord serum samples. The median concentration of  $\Sigma(4)$ OH-BDEs was 33 pg/mL (17-231) in maternal serum and 43 pg/mL (23-446 pg/mL) in cord serum samples. The differences between maternal and cord blood concentrations were statistically significant by Wilcoxon signed rank test. The PBDEs and OH-PBDEs measured in cord serum were higher than in paired maternal serum samples. This suggests fetuses may receive higher PBDE and OH-PBDE exposure compared with their mothers.

**457 Distribution of PCBs and Their Hydroxylated Metabolites in Human Serum from Asian Countries** A. Eguchi, Ehime Univ, Center for Marine Environmental Studies, Ehime Univ, Center for Marine Environmental Studies (CMES), Ehime Univ, Center for Marine Environmental Studies Ehime Univ; K. Nomiya, Ehime Univ, Center for Marine Environmental Studies (CMES); G. DEVANATHAN, Ehime Univ, Center for Marine Environmental Studies (CMES), Ehime University, Center for Marine Environmental Studies; A. Subramanian, Ehime Univ, Center for Marine Environmental Studies (CMES); P.H. Viet, Hanoi National Univ; P. Parthasarathy, E-parisara (P) Ltd; K.A. Bulbul, Nijalingappa College; S. Takahashi, Ehime Univ, Center for Marine Environmental Studies (CMES); S. Tanabe, Ehime Univ, Center for Marine Environmental Studies. Polychlorinated biphenyl congeners (PCBs) are known to affect endocrine systems and neurodevelopment in human and wildlife. Even though PCBs were banned in many countries in the late 1970s, they still dominate the body burden of organohalogen compounds in biota including human. In this study, we investigated the levels and profiles of PCBs and hydroxylated metabolites of PCBs (OH-PCBs) in human serum samples collected from

three Asian countries, Japan ( $n = 51$ ), India ( $n = 42$ ), and Vietnam ( $n = 30$ ). Median concentrations of PCBs in serum from Japanese cancer patients (2900 pg g<sup>-1</sup> wet wt) were higher than Indian e-waste recycling workers and residents near coastal area (70 and 110 pg g<sup>-1</sup> wet wt, respectively) and also than Vietnamese e-waste recycling workers and rural residents (300 and 400 pg g<sup>-1</sup> wet wt, respectively). Median concentrations of OH-PCBs were also higher in Japanese serum (630 pg g<sup>-1</sup>) samples than other locations, indicating the effects of past higher consumption of PCBs and food habits in Japan. In general, CB105, CB118, CB138, CB153 and CB180 contributed high proportion to the total PCBs. In all locations, 4-OH-CB107, 4-OH-CB146 and 4-OH-CB187 dominated the OH-PCB profiles in serum samples. However, high contributions of CB28, CB74 and 4OH-CB79 in e-waste recycling workers and the difference in PCBs and OH-PCBs pattern between e-waste recycling workers and general population might be due to the presence of specific exposure sources in e-waste recycling locations. Parent congener for 4OH-CB79 is not clear, but higher concentrations of lower chlorinated PCBs may be a source of lower chlorinated OH-PCBs in e-waste recycling workers in India.

**458 Spatial and Phase Distribution of PCBs, PBDEs and Their Metabolites in Air and Soil, Korea** U. Kim, S. Oh, H. Kim, Pusan National Univ; S. Choi, Ulsan National Institute for Science and Technology; H. Moon, Hanyang Univ; J. Oh, Pusan National Univ. This paper reports concentration level, spatial and phase distribution of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and their metabolites in air and soil. Although there has been quite a lot of research done for persistent organic pollutants level, distribution, physicochemical properties in environmental matrices, still only a few of information was secured about PCBs or PBDEs existed in air phase and much less data was available for their metabolites in various type of environmental matrices. Especially in case of Korea, data of PCBs and PBDEs were not sufficient compared to other POPs like PCDD/Fs. Therefore, in this study, 20 surface soil and 14 air samples were collected from December, 2010 to July, 2011 with high volume sampler (HV-1000R, Sibata, Japan) and SPMD passive air samplers. To examine spatial distribution, sampling points were selected to cover industrial, residential and rural sites in Busan, south-eastern city of Korea. Basic information including temperature, wind velocity, humidity of sampling points was also recorded. Total PCBs and PBDEs were extracted with soxhlet, cleaned up with multi-silica or followed by alumina column and measured by HRGC/HRMS for PBDEs and LRGC/LRMS for PCBs. For hydroxylated and methoxylated metabolite analysis, PCBs and PBDEs were derivatized by methylation and acetylation, respectively, and analysed by HRGC/HRMS. For air samples, gas phase and particle phase were analysed separately. Recoveries of PCBs were 60-114% and 36-148% for PBDEs, satisfying EPA standards. The concentration level of PCBs in soil was ranged from

**459 Competitive Binding of Brominated Flame Retardants, Metabolites and Halogenated Phenolics with Thyroxine to Human Thyroxine-Binding Globulin** C. Butt, H.M. Stapleton, Duke Univ, Nicholas School of the Environment. Thyroid hormones (THs) are essential for normal physical and mental development in humans. THs are produced in the thyroid gland, primarily as the pro-hormone thyroxine (T<sub>4</sub>) in addition to minor amounts of the biologically active 3,3',5'-triiodothyronine (T<sub>3</sub>). Only a small fraction (< 1%) of circulating THs are "free" or unbound, and the vast majority of circulating THs are bound to plasma proteins. The plasma proteins include serum albumin, transthyretin (TTR) and thyroxine-binding globulin (TBG). TBG has the highest affinity for T<sub>4</sub> and binds approximately 75% of serum T<sub>4</sub>. It has been shown that some halogenated phenolics can competitively bind with TBG and TTR transport proteins, thus displacing T<sub>4</sub>. Whereas the competitive binding to TTR is fairly well studied, there is comparatively less known about TBG. This study used in vitro techniques to investigate competitive binding to TBG by several halogenated phenolic compounds. Briefly, <sup>13</sup>C<sub>12</sub>-T<sub>4</sub> was combined with human TBG in Tris-HCl buffer and various concentrations of competitors were spiked. Competitors included tetrabromobisphenol A (TBBPA), 2,4,6-tri-bromophenol (2,4,6-TBP) and triclosan. T<sub>4</sub> was used as the reference compound. Control samples consisted of clean DMSO. After overnight incubation, TGB-bound <sup>13</sup>C<sub>12</sub>-T<sub>4</sub> was separated from "free" <sup>13</sup>C<sub>12</sub>-T<sub>4</sub> using mini-size exclusion chromatography columns as previously described. The TGB-bound <sup>13</sup>C<sub>12</sub>-T<sub>4</sub> was analyzed by LC-MS/MS using methods previously developed by our laboratory. All three competitors tested (TBBPA,

2,4,6-TBP and triclosan) showed the ability to competitively bind to TBG in a dose-response manner. Calculated IC<sub>50</sub> values were 0.22 μM, 20 μM and 149 μM for T<sub>4</sub>, TBBPA and triclosan, respectively. Competitive binding for 2,4,6-TBP was only observed at the highest dose and regression parameters could not be calculated. The corresponding relative potency values for TBBPA and triclosan were 0.01 and 0.001, respectively. The present study demonstrated that two brominated flame retardants as well as the anti-microbial, triclosan, can compete with T<sub>4</sub> for binding to TBG. This study provides further evidence that some halogenated phenolics can competitively bind with TBG and thus may impact the circulation of THs, particularly T<sub>4</sub>, throughout the human body. Future work will investigate additional flame retardants and their metabolites (i.e., OH-BDEs).

**460 Compound-specific Isotope Analysis of Halogenated 1'-methyl-1,2'-bipyrroles Narrows the Search for Their Biosynthetic Origin** K. Pangallo, Colgate Univ, Chemistry; C.M. Reddy, Woods Hole Oceanographic Institution; J. Bolotin, T. Hofstetter, Eawag, Environmental Chemistry. Highly halogenated 1'-methyl-1,2'-bipyrroles are natural products that biomagnify into the upper trophic levels of the marine food web. Though they have been found throughout the world's oceans, the organism or organisms responsible for their biosynthesis remains unknown. One way to elucidate the origin of these compounds is through the isotopic signature imprinted during biosynthesis and maintained during biomagnification (we will argue it is not due to degradation). Here we use compound-specific nitrogen isotope analysis to measure this signature, and demonstrate that they are dramatically enriched in δ<sup>15</sup>N. This <sup>15</sup>N-enrichment is greater than that seen for other biosynthetic organic compounds measured to date, and is a distinct isotopic signature. By interpreting this result in the context of strongly fractionating biochemical pathways and enriched precursors, we suggest that future research on the origin of these compounds may want to focus on marine actinomycetes and/or *Pseudoalteromonas*.

**461 Halogenated By-products of Gemfibrozil Formed in Wastewater Treatment-like Conditions: In Situ Characterization, Detection, and Biological Effects** D. Bulloch, Univ of California Riverside; R. Lavado, Univ of California Riverside, Dept of Environmental Sciences; C.K. Larive, Univ of California Riverside, Dept of Chemistry; D. Schlenk, Univ of California Riverside, Dept of Environmental Sciences. The study of disinfection by-products of pharmaceuticals in wastewater is an emerging field. Most by-products of known contaminants are unregulated, and may have altered physicochemical properties and toxicity profiles compared with the parent. The lipid regulator gemfibrozil has been found consistently in the high ng/L to low μg/L range in wastewater in Southern California. Gemfibrozil can be oxidized when treated with sodium hypochlorite in deionized water to form a *p*-chlorinated species as the major product. When treated with sodium hypochlorite in wastewater, a *p*-brominated species also forms as a major product. Product evolution and distribution during chlorination reactions were monitored using a Waters Acquity ultra performance liquid chromatography with time-of-flight mass spectrometric detection. Both halogenated analogs of gemfibrozil were purified by HPLC, where subsequent structural assignments were carried out by one- and two-dimensional nuclear magnetic resonance on a Bruker Avance 600. The detection of gemfibrozil and its halogenated analogs was made using chlorinated primary effluent from a Southern California wastewater treatment plant. Measureable contaminant concentrations were determined in the effluent samples for gemfibrozil, chlorinated gemfibrozil, and brominated gemfibrozil. Characterization of the biological activity of gemfibrozil and its halogenated analogs was performed through in vitro experiments in rainbow trout primary hepatocytes at concentrations up to 100 μg/L, where no activity was observed. Biological activity characterization in vivo in a 14-day waterborne exposure using Japanese medaka yielded reduced 11-keto testosterone levels in whole fish, where the halogenated analogs elicited an enhanced anti-androgenic response compared to the parent gemfibrozil. The levels of 17-β estradiol were also measured in the whole fish, where the ratio of 11-keto testosterone to 17-β estradiol revealed a trend of enhanced endocrine disruption for gemfibrozil's halogenated analogs when compared to the parent gemfibrozil.

**462 Genomic Tools for Understanding Chemical Tolerance in a Wild Population of the Estuarine Fish, *Fundulus heteroclitus*** D. Proestou, US Environmental Protection Agency, Atlantic Ecology Division; J. Martinson, E. Waits, US Environmental Protection Agency, Ecological Exposure Research Division; D. Champlin, US Environmental Protection Agency,



Atlantic Ecology Division; D. Nacci, US Environmental Protection Agency. Wild populations of the killifish *Fundulus heteroclitus* residing in heavily contaminated North American Atlantic coast estuaries have recently and independently evolved dramatic, heritable, and adaptive polychlorinated biphenyl (PCB) tolerance. However, currently available genomic tools limit our ability to characterize the genetic and biochemical mechanisms associated with PCB tolerance in this species. In order to enhance ongoing research designed to reveal the genetic basis for PCB tolerance, we focused on genes associated with the aryl hydrocarbon receptor (AHR) putative target pathway and developed a suite of Single Nucleotide Polymorphism (SNP) markers. Expressed Sequence Tag (EST) sequences derived from > 100 genes of interest were mined from the *F. heteroclitus* EST database and assembled into 155 contigs using the sequence assembly program CAP3. The QualitySNP pipeline, an algorithm designed to detect polymorphisms in EST data, was used to identify 'true' SNPs within contigs. Over 400 SNPs were detected and from those, 120 markers generated. Eight fish, which differed in PCB sensitivity and represent the parental generation of 4 mapping families, were genotyped at each SNP locus using a melting temperature (T<sub>m</sub>)-shift method. Genotype data were used to determine the utility of each marker in genetic map construction and Quantitative Trait Locus (QTL) analysis. A subset of these markers is currently being evaluated for its application in population genetic studies. This research is a reflection of the need for developing genomic tools in wild, ecologically-relevant species to better understand stress response and evolved adaptive differences among wild populations.

**463 Cytochrome P4501A Promoter Polymorphisms and Activity in Natural Populations** L.M. Williams, Woods Hole Oceanographic Institution, Biology; M.E. Oleksiak, Rosenstiel School of Marine & Atmospheric Sciences. Cytochrome P4501A (CYP1A) induction is a widely used biomarker of aromatic hydrocarbon exposure. Unexpectedly, CYP1A is refractory to prototypic inducers in multiple populations of the estuarine minnow, *Fundulus heteroclitus*, adapted to chronic anthropogenic pollutants. In these polluted populations, two single nucleotide polymorphisms (SNPs) in the promoter and first intron of CYP1A are under selection, indicating that natural selection is acting on the CYP1A promoter or loci linked to these SNPs. To better understand the role of these selectively important SNPs in CYP1A transcriptional regulation, 1,630 bp of the CYP1A promoter and first intron and exon were sequenced in eight individuals from each of three populations: a polluted (New Bedford Harbor, MA USA) population resistant to aromatic hydrocarbons and two sensitive, reference populations north and south of the polluted site. The CYP1A promoter was extremely variable (an average of 9.3% of the promoter nucleotides varied among all populations) and exhibited no fixed differences between populations. In vitro, a prototypic polycyclic aromatic hydrocarbon (PAH) induced transcription from the CYP1A promoter in a dose-dependent manner for both polluted New Bedford Harbor and reference population individuals. When promoters from four individuals per population were tested, transcription levels driven by CYP1A promoters from New Bedford Harbor individuals were significantly greater than those for reference individuals. This is in contrast to CYP1A expression in vivo, which is refractory to induction in New Bedford Harbor individuals. These data suggest that the nucleotide variation in the CYP1A promoter affects the PAH induced transcription, but to fully explain the pattern of expression in natural populations, other cis-acting or trans-acting factors that act through the selectively important SNP must also be involved.

**464 The Role of Differential Metabolism and DNA-adduct Formation in the Resistance of Atlantic Killifish (*Fundulus heteroclitus*) to Cancer** B.W. Clark, Duke Univ, Nicholas School of the Environment ; N.R. Herr, Univ of North Carolina at Chapel Hill, Dept of Environmental Sciences and Engineering; E.M. Cooper, Duke Univ, Nicholas School of the Environment ; L.B. Collins, Univ of North Carolina at Chapel Hill, Dept of Environmental Sciences and Engineering; B.C. Moeller, Univ of North Carolina at Chapel Hill, Dept of Environmental Sciences and Engineering, Curriculum of Toxicology; H.M. Stapleton, Duke Univ, Nicholas School of the Environment ; J.A. Swenberg, Univ of North Carolina at Chapel Hill, Dept of Environmental Sciences and Engineering, Curriculum of Toxicology; R. Di Giulio, Duke Univ, Nicholas School of the Environment. The Elizabeth River (ER) estuary is an urbanized and industrialized watershed at the south end of the Chesapeake Bay. The Atlantic Wood Industries Superfund Site (Portsmouth, VA, USA) on the ER is highly contaminated

with creosote-derived polycyclic aromatic hydrocarbons (PAHs). Atlantic killifish (*Fundulus heteroclitus*) inhabiting the site are resistant to the acute toxicity and cardiac teratogenesis caused by some PAHs and PCB-126 (3,3',4,4',5-pentachlorobiphenyl). The resistance is linked to down regulation of the aryl hydrocarbon receptor (AHR) pathway, including recalcitrance to induction of cytochrome P450s. However, adult ER killifish show a greater prevalence of hepatic and pancreatic tumors compared with those from reference sites (Vogelbein and Unger, 2006). Despite the prevalence of cancer in wild-caught ER killifish, we recently demonstrated that they are markedly resistant to benzo[a]pyrene (BaP)-induced DNA damage and chronic hepatic toxicity, including cancer. We hypothesize that ER killifish are protected by down-regulation of the AHR pathway, along with upregulation of phase II and III metabolism and antioxidant defense systems. The objective of the current research was to determine the role of differential BaP metabolism and DNA adduct formation in this resistance. We developed an LC-MS/MS-SRM method to simultaneously and quantitatively monitor the benzo[a]pyrene diol epoxide (BPDE) stable DNA adduct and the oxidative-stress-related adduct, 8-hydroxy-2'-deoxyguanosine (8-oxo-dG), generated from base oxidation. We also measured the occurrence of abasic sites, which can be indicative of oxidative DNA damage. In addition, we refined methods for the assessment of the major BaP metabolites in liver and bile. In the current study, adult ER and King's Creek (KC, reference population) killifish were injected intraperitoneally with 10 mg/kg BaP, and liver and bile were harvested at various timepoints following exposure. We compared the levels of BPDE-dG adducts, 8-oxo-dG adducts, and abasic sites in the two populations. These results were correlated with differences in the levels of BaP and its metabolites measured in liver and bile. In addition, we compared mRNA expression of glutathione S-transferase-*mu* and the ABC transporters ABCB1 and ABCB11. This work was funded by the NIEHS-supported Superfund Research Program (P42ES10356 and P42ES010356-09S3, ARRA Supplement) and the Duke Integrated Toxicology and Environmental Health Program (T32ES07031).

**465 Development and Application of Genomic Resources for *Fundulus heteroclitus* Reveal Environment Dependent Arsenic by Gene Interactions** J.R. Shaw, Indiana Univ, The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics, Dartmouth College, Dept of Biology; T. Hampton, Dartmouth Medical School; J.K. Colbourne, Indiana Univ, The Center for Genomics and Bioinformatics; C.Y. Chen, Dartmouth College; B.A. Stanton, Dartmouth Medical School. The killifish, *Fundulus heteroclitus*, is a phenotypically plastic, ecologically diverse, and environmentally tractable euryhaline teleost that has been widely used as both a surrogate for studies of human health and a sentinel monitor for environmental quality. It is well positioned to become a model system for environmental genomics—which seeks to understand how gene function is influenced by environmental conditions, while accounting for variation that exists within and among natural populations. To facilitate such applications, our group has been working to develop genomic resources for killifish. In the present study, we detail our efforts to define its transcriptome and develop an ultra-dense microarray. We highlight the application of these tools through functional studies on the effects of arsenic during seawater acclimation. Next generation sequencing was used to characterize the killifish transcriptome illuminated under 72 different conditions, including arsenic and salinity. The ~1,500,000 sequence reads were assembled, computationally annotated, and used to develop a microarray on the NimbleGen 12-plex platform (12 sub-arrays/slide, each containing 137,000 probes). For the functional studies, killifish were acclimated to freshwater and the transcriptomic response was measured as a function of salinity, time and arsenic exposure (100 ppb) following immediate transfer to seawater. These investigations employed a replicated, factorial design and differentially regulated genes were determined by fit to a linear model. Arsenic constrains physiological plasticity by inhibiting seawater acclimation and this was captured in the transcriptomic response, where gene-response profiles obtained following 1-h and 24-h in seawater in the presence of arsenic more closely resembled those of freshwater fish. Not surprisingly these included genes known to be involved in immune function, cellular organization and remodeling, cell signaling, ion transport, and arsenic, as well as, many novel genes. Perhaps the most striking finding was the emergence of a large interactive transcriptome—gene responses not predicted by simple addition of their activity in arsenic or seawater alone. These responses represent environment dependent gene by arsenic interactions, and highlight the critical

need for environmentally responsive model systems in defining critical toxicity pathways that influence natural populations, which live in variable environments.

**466 The Role of Aryl Hydrocarbon Receptor (AHR) Variants in the Mechanism of Evolved Resistance to PCBs in Populations of *Fundulus heteroclitus*** M.E. Hahn, Woods Hole Oceanographic Institution, Biology Dept; S.I. Karchner, A.M. Reitzel, Woods Hole Oceanographic Institution, Biology; M.J. Jenny, Univ of Alabama; D.G. Franks, Woods Hole Oceanographic Institution; D. Nacci, USEPA, ORD, NHEERL, Atlantic Ecology Division, USEPA, NHEERL, Atlantic Ecology Division; M.F. Oleksiak, Rosenstiel School of Marine & Atmospheric Sciences; D.B. Mark Welch, Marine Biological Laboratory; I. Wirgin, New York Univ School of Medicine, Dept of Environmental Medicine. Long-term exposure to polychlorinated biphenyls (PCBs) and other aromatic hydrocarbons has selected for populations of Atlantic killifish (*Fundulus heteroclitus*) with evolved resistance to non-*ortho*-substituted PCBs and other dioxin-like compounds that act via the aryl hydrocarbon receptor (AHR). Analysis of gene expression profiles by microarray and RNA-Seq suggest a genome-wide loss of responsiveness to AHR agonists in fish inhabiting the New Bedford Harbor, MA (NBH) Superfund site as compared to fish from Scorton Creek, MA (SC). We are studying the relative roles of aryl hydrocarbon receptor (AHR) duplicates (paralogs) and polymorphic variants of AHR1, AHR2, and AHR repressor (AHRR) in controlling the differences in sensitivity to PCBs in these populations. *Fundulus* AHR loci are highly polymorphic and display gene- and site-specific patterns of haplotype diversity and evidence of selection, especially at the AHR2 locus. However, the haplotype differences have not yet been linked to differences in AHR function that can be measured in vitro. In PCB-resistant tomcod (*Microgadus tomcod*) from the Hudson River, PCB resistance is caused by a 2-amino-acid deletion in one of two AHR2 variants, resulting in reduced binding affinity for TCDD. In contrast, killifish exhibit 26 AHR2 variants with no measurable differences in binding affinity. Deep sequencing of the *Fundulus* transcriptome revealed two additional AHR paralogs (AHR1b, AHR2b) that also might have roles in the evolved resistance. [Supported by P42ES007381, R01ES006272, and the Smith Chair.]

**467 Mechanistic Basis of Resistance to PCBs and Dioxin in Atlantic Tomcod from the Hudson River** I. Wirgin, New York Univ School of Medicine, Dept of Environmental Medicine; N.K. Roy, New York Univ School of Medicine, Environmental Medicine; M. Loftus, New York Univ School of Medicine; R.C. Chambers, NOAA, Northeast Fisheries Science Center; D.G. Franks, Woods Hole Oceanographic Institution, Biology; M.E. Hahn, Woods Hole Oceanographic Institution. The main stem Hudson River (HR) has the largest federal Superfund site in the US because of contamination with PCBs and the western HR estuary also contains a Superfund site because of contamination with near record levels of TCDD. We have shown that Atlantic tomcod from the main stem HR and Newark Bay bioaccumulate high hepatic levels of coplanar PCBs and TCDD, respectively, compared to tomcod from elsewhere. Atlantic tomcod from the Hudson River are highly resistant to PCBs and TCDD-induced early life-stage toxicities and induction of cytochrome P4501A, responses that are mediated by the aryl hydrocarbon receptor (AHR) pathway. We investigated the molecular basis of this resistance in tomcod. AHR2 sequence was highly conserved among tomcod populations coastwide. However, tomcod from the main stem HR and the Hackensack River (tributary of Newark Bay) exhibited two non-synonymous genetic polymorphisms (one six base deletion and one SNP) in AHR2 (the predominant form of AHR in fishes) that were nearly absent in tomcod from six other Atlantic Coast estuaries including the two in closest proximity to the HR. In contrast, haplotype frequencies at the selectively neutral mtDNA control region were significantly correlated with geographic distance indicating that the genetic dissimilarity of the Hudson River population at functionally important AHR2 was not observed at a selectively neutral locus. The variant AHR2 proteins were in vitro expressed and compared for their functional significance in two assays. In ligand binding assays, the HR-specific AHR2-1 protein was impaired compared to the more common AHR2-2 protein in binding TCDD. The HR AHR2-1 protein was also less able to drive gene expression in transient transfection assays in AHR deficient mammalian cells treated with TCDD or PCB126 than the more common AHR2-2 protein. Additional transfections with recombinant AHR2s demonstrated that the six-base deletion in AHR2 is the mechanistic basis of resistance. We are now attempting to

chronicle the timing of the onset of resistance in the HR population using tomcod archived in museum collections. Our results show that the HR tomcod population has undergone rapid evolutionary change probably due to contaminant exposure. This is the first demonstration of the mechanistic basis of resistance to contaminants in a vertebrate population and shows that rapid evolutionary change can result from one variant in a single gene.

**468 Evidence of Metal Effects in Wild Yellow Perch Using Biometric, Biochemical, Physiological and Molecular Approaches** P. Couture, INRS, Centre Eau Terre Environnement; P.G. Campbell, INRS-Eau, Terre et Environnement, INRS-ETE, , INRS, Centre Eau Terre Environnement; L. Bernatchez, Université Laval, Département de Biologie; F. Pierron, CNRS. Metal accumulation and toxicity to fish has been extensively studied in the laboratory. In the field, however, fish living in metal-contaminated sites have typically lived under conditions of low-level, chronic metal exposure for several generations and may have adapted to some extent to metal stress. Furthermore, variations in natural factors like temperature, food availability, competition and predation may diminish or exacerbate metal toxicity, making it difficult to discriminate metal effects on fish health from natural variations. The yellow perch (*Perca flavescens*) is ubiquitous in North American freshwaters and is particularly abundant in areas of Canada where intense metal mining and smelting activities take place. With over 50 publications examining metal accumulation and effects in this species, conducted mostly in the mining regions of Sudbury (Ontario) and Rouyn-Noranda (Québec) where the contaminants of highest concern are Cd, Cu and Ni, the yellow perch is starting to emerge as an ideal model species for the investigation of metal effects in wild fish. These studies have demonstrated that metal-contaminated wild yellow perch face energetic constraints yielding to lower aerobic capacities (as indicated by enzyme activities, swim performance and respiration rates), growth rate and energy accumulation. The expression level of genes encoding for proteins involved in metal detoxification (metallothioneins), protein protection (heat shock protein-70), growth (insulin-like growth factor-1), aerobic energy metabolism (cytochrome c oxidase, cco-1) and protection against oxidative stress (Cu/Zn superoxide dismutase) and the activity of the CCO enzyme were affected by metal contamination. Using the 454 sequencing technology, we reported that exposure to Cd and Cu was associated with a decrease in the transcription levels of numerous genes involved in protein biosynthesis, in the immune system and in lipid and energy metabolism. The recent development of a 1000-gene microarray opens up further research opportunities. A population genetics study revealed that long-term metal contamination can also yield to bottlenecks and decreased genetic diversity. Indirect (food-web induced) metal effects and natural, seasonal variations in health indicators have also been demonstrated. We will review available evidence that a combination of biometric, biochemical and molecular tools can help discriminate metal effects from natural factors in wild yellow perch.

**469 Bisphenol A Modulates Expression of Sex Differentiation Genes in the Self-fertilizing Fish, *Kryptolebias marmoratus*** J. Lee, Hanyang Univ, Dept of Chemistry, Hanyang Univ Graduate School, Dept of Chemistry; Y. Lee, Sangmyung Univ, Dept of Green Life Science. Endocrine disrupting chemicals (EDCs) have been a major concern in the normal reproduction and development of aquatic organisms. In the teleost, steroid hormones are synthesized via the steroidogenesis pathway, and play a key physiological role in the regulation of gonadal sex differentiation. The protogynous hermaphroditic fish, *Kryptolebias marmoratus* is the only vertebrate capable of reproducing through internal self-fertilization. To uncover the effect of bisphenol A (BPA) on sex differentiation genes on transcription, we investigated the expression patterns of several sex differentiation-related genes such as *dax1*, *dmrt1*, *mis*, *sf1*, *figl*, *StAR* and *wt1* after BPA exposure with controls (E2 and TMX). In response to 17-estradiol (E2) exposure, a testis-specific gene, *dmrt1* mRNA was down-regulated in the gonad of the secondary male but the expression of the female-specific gene, *dax1* mRNA was significantly elevated in the brain and gonad. A high level of *StAR* mRNA was detected in the brain and gonad of both hermaphrodite and secondary males, suggesting that the elevated expression of *dax1* and *StAR* genes would be involved in E2 exposure. As expected, upon BPA exposure, the *dmrt1* and *MIS* mRNA level decreased in both hermaphrodite and secondary males, while the female-specific gene, *figl* mRNA level increased in the gonad of both genders. BPA showed an opposite mode of action on the expression of *dax1* (induction,  $P > 0.05$ ) and *sf1* mRNA (inhibition,  $P > 0.05$ ) in the brain and gonad against both genders. The sensitivity of *dax1*

to BPA on expression was relatively high in the secondary male. The wt1 mRNA was up-regulated in most tissues except in the liver of BPA-exposed secondary males. Regarding the time course study, the fig1 mRNA level increased at 6 h after BPA exposure. In addition, BPA elevated the expression of StAR, dax1, and wt1 mRNA but repressed sf1 mRNA. In this paper, we demonstrated that BPA may modulate the expression of sex differentiation and steroidogenesis pathway genes, and this finding would provide a better understanding on the modulation of transcription upon BPA exposure in steroidogenesis and sex differentiation in the hermaphroditic fish, *K. marmoratus*.

#### 470 Determining the Relative Phytotoxicity of Rapidly Dewatered Oil Sands Tailings

**J. Cutter**, J. Germida, Univ of Saskatchewan; S.A. Armstrong, Shell Health; J. Reid, Shell Canada; B. Fahlman, K. Peru, J. Headley, Environment Canada. Atmospheric fines drying (AFD) is a new tailings treatment strategy being piloted at Shell Canada's Muskeg River Mine near Fort McMurray, Alberta. The AFD process involves adding a polyacrylamide polymer to the tailings which are then thinly spread over a sloped disposal area to rapidly dewater. Hydroponic experiments using cattail (*Typha latifolia*) and common reed (*Phragmites australis*) were conducted to evaluate the relative phytotoxicity of AFD release and runoff waters in comparison to the reclaim water of traditional tailings deposits. Plant health was assessed by examining water uptake and fresh weight gain. Significant differences were found between the transpiration of control plants and plants grown in tailings water however, no statistically significant differences were found between the waters associated with different tailings treatments (traditional reclaim and AFD) on the effect of water uptake ( $\alpha=0.05$ ). Significant differences were also found between the fresh weight gain of control cattail and cattail subjected to the different tailings treatments ( $\alpha=0.05$ ) but not between treatments of MFT and AFD waters. As naphthenic acids have been identified to contribute to the toxicity of oil sands tailings, they were isolated and investigated for differences in these two types of waters. Naphthenic acids were monitored at the start and end of each experiment to test for any biodegradation or plant uptake. Results indicate that AFD release waters have a similar relative toxicity as the reclaim waters from MFT tailings and therefore will not likely increase the environmental hazard of oil sands tailings.

#### 471 Comparison of Ozonation and Reclamation Pond Biodegradation as Treatments to Eliminate Oil Sands Process Water Toxicity in *Chironomus dilutus*

**J. Anderson**, S.B. Wiseman, Univ of Saskatchewan, Toxicology Centre; N. Wang, Univ of Alberta, Dept of Civil and Environmental Engineering; L.A. Perez, Univ of Alberta, Div. Analytical & Environmental Toxicology; M.G. Eldin, Univ of Alberta, Dept of Civil and Environmental Engineering; J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine and Pathology; K. Liber, Univ of Saskatchewan, Toxicology Centre; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science. Global energy demands are driving a shift from conventional oil sources to alternatives such as oil sands. Canada hosts the second largest oil reserves, most of which is located within the Alberta oil sands. Bitumen extraction using hot water and caustic soda results in oil sands process water (OSPW). Over a billion m<sup>3</sup> of OSPW are held in settling ponds and this volume will increase due to a zero-discharge policy. OSPW is saline, alkaline, and contains high naphthenic acid (NA) concentrations. NAs are a group of carboxylic acids implicated as the primary cause for acute and chronic toxicity of OSPW. Several different approaches have been used in experimental reclamation ponds in an effort to eliminate OSPW toxicity by reducing NA concentrations via biodegradation. Ozonation has also been proposed for treatment of OSPW. To compare the effectiveness of natural aging and ozonation as methods of reducing the toxicity of OSPW, studies were conducted using *C. dilutus*, a model benthic invertebrate. Water was sampled from 3 reclamation ponds: Big Pit (mature fine tailings (MFT) capped by freshwater in 1993), FE5 (MFT capped with OSPW in 1989), and TPW (OSPW aged since 1993). In addition, relatively 'fresh' OSPW was collected from the West In-Pit (WIP) and was ozonated with 30 mg/L or 80mg/L O<sub>3</sub>. Both a 10-day and long-term exposure assay were conducted using 8-9 day old larvae. Following the 10-day exposure, there were no differences between growth and survival in any of the reclamation pond waters compared to freshwater controls. WIP-exposed larvae were 64-77% smaller than the controls ( $p < 0.05$ ), while ozonation of WIP attenuated growth inhibition. After chronic exposure, there was significantly less pupation in the FE5 group compared

to the controls ( $p < 0.05$ ). Exposure to untreated OSPW delayed male pupation ( $p < 0.01$ ) and reduced adult emergence ( $p < 0.05$ ), both of which were improved by ozonation. Emergence was significantly impaired in TPW ( $p < 0.01$ ), with delays in both male and female emergence ( $p < 0.05$ ). Females were also delayed in the Big Pit treatment ( $p < 0.05$ ). These results suggest that in spite of reduced NA concentrations in reclamation ponds, there is still residual chronic toxicity of OSPW that impacts *C. dilutus* development and reproduction. Ozonation attenuates growth and reproductive effects and may be beneficial in combination with biodegradation as aging alone is not sufficient to eliminate OSPW toxicity.

#### 472 In Vivo and In Vitro Bioassays for the Evaluation of OSPW Immunotoxicity: Potential Tools for Evaluating OSPW Remediation Processes

**E. Garcia-Garcia**, Univ of Alberta, Biological Sciences; J. Ge, A. Oladiran, Univ of Alberta, Dept of Biological Sciences; M.G. Din, Univ of Alberta, Dept of Civil and Environmental Engineering; L.C. Perez-Estrada, J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine and Pathology; M. Belosevic, Univ of Alberta. Large volumes of toxic aqueous tailings are produced in northern Alberta by the oil sands industry. Naphthenic acids (NAs) are thought to be the major toxic component in Oil Sands Process Water (OSPW), according to bacteria-based toxicological assays. In addition to NAs, OSPW organic fraction contains a complex mixture of compounds that may also be toxic particularly to multicellular organisms. We developed in vitro and in vivo bioassays to evaluate the toxicity of OSPW organic fraction in mice. A popular commercial NAs preparation from Merichem Chemicals was found not to be an adequate model for the study of OSPW NAs toxicity, as it had very different effects on mouse immune mechanisms, compared to the extracted organic fraction of OSPW (OSPW-OF), containing equivalents NAs amounts. Ozonation has recently been used as an efficient method for degrading OSPW NAs and decreasing its toxicity towards bacteria. We tested whether ozonation reduced OSPW immunotoxicity, through the degradation of NAs, and other unidentified OSPW contaminants potentially involved in the observed alterations in immune responses of mice. In vitro exposure to OSPW-OF decreased the ability of bone marrow-derived macrophages (BMDM) to produce nitric oxide, reactive oxygen intermediates, and decreased the ability of macrophages to engulf particles. Decreased nitric oxide and reactive oxygen intermediate production was related to down-regulation of gene expression of the inducible nitric oxide synthase and NADPH oxidase subunits, respectively. OSPW-OF also altered gene expression of pro-inflammatory cytokines in BMDM. Oral administration of OSPW-OF to mice altered gene expression of pro-inflammatory cytokines and chemokines in the mesenteric lymph nodes and the liver. OSPW ozonation, before extraction of the organic fraction, completely abrogated the effects of OSPW-OF in vitro, and returned the expression of most cytokine genes to control levels in vivo. Our results indicate that ozonation may be a valuable treatment process for the remediation of the large volumes of water in oil sands tailing ponds. The in vitro and in vivo assays developed in this study will be valuable tools for the evaluation of different OSPW remediation regimens.

#### 473 A New Integrative Monitoring Program for the Canadian Oil Sands

**E.J. Wrona**, P.D. di Cenzo, D. Wicklum, Environment Canada; P. McEachern, Alberta Environment; K. Schaefer, Environment Canada. The Canadian Oil Sands, which comprise 97% of Canada's 176 billion barrels of proven oil reserves, are located beneath 140,200 km<sup>2</sup> of boreal forests, prairies and wetlands, and are the second largest known deposit of crude oil in the world. Presently, the resources being recovered are located in north-eastern Alberta in three main regions: Athabasca, Cold Lake, and Peace River. The Athabasca River and tributaries flow through the oil sands deposits, eroding the oil-containing sands and interacting with groundwater systems also in contact with the oil formations. Primary environmental concerns regarding water quality, quantity and ecosystem health related to oil sands development are within the lower portions of the Athabasca River catchment, primarily downstream of Fort McMurray, Alberta, although they are potentially farther reaching. Within the Lower Athabasca basin, and in particular in the oil sands region, both "pulse" and "press" environmental stressors, and related impacts, are occurring. These affect both the temporal and spatial variability in observed responses and influence the spatial and temporal replication necessary to detect impacts with sufficient statistical power. In response to criticisms of current monitoring activities in the Canadian Oil Sands region, the Canadian Minister of the Environment committed Environment Canada to lead, in collaboration with Alberta



Environment and relevant stakeholders, the development and implementation of a new, integrative monitoring program for the oil sands region. A new monitoring program has been developed, utilizing an ecosystem-based, integrative, mass-balance approach, which builds upon and integrates existing monitoring efforts into an adaptive, and holistic system. The program design is adaptive – alterations in spatial coverage and temporal sampling frequency is linked to science-based (and policy-relevant) decision-triggers. A key objective is to maintain required statistical rigour to understand, predict and report on the status and trends of water quality and quantity, accumulated state, changes in ecosystem structure, function and health and ultimately determine cumulative impacts. The program design will be continually assessed and refined based on stakeholder feedback and new science findings. This presentation provides a scientific and technical overview of the new program and highlights key challenges and steps in its implementation.

**474 Development of a Comprehensive Aquatic Biomonitoring Program for Canada's Oil Sands Areas** J.L. Parrott, Environment Canada, Environment Canada, National Water Research Institute, Environment Canada; M.E. McMaster, Environment Canada, National Water Research Institute; F. Wrona, P. di Cenzo, N. Glozier, J.M. Culp, Environment Canada. Canada's Minister of the Environment committed Environment Canada to lead, in collaboration with Government of Alberta and relevant stakeholders, the development of a preliminary surface water quality, biodiversity and effects monitoring plan for the lower Athabasca River. The biodiversity and effects monitoring component includes recommendations for the sampling of multiple trophic levels in the main stem of the Athabasca River, its major tributaries, and smaller streams in the area between Fort McMurray and Wood Buffalo National Park Boundary. The Fish monitoring program consists of assessment of sentient adult fish to determine if there are differences in the growth, reproduction, survival or condition of fish. Young of year fish will also be assessed on tributaries, to check for recruitment and growth effects. As well, fish communities will be surveyed at selected sites in order to assess potential changes over time and to understand community structure. The plan will also monitor concentrations of contaminants in fish tissue as an indicator of fish exposure and human usability. The proposed design for assessing ecological effects on benthic biota will include community assessments of both the algae and macroinvertebrates associated with the benthic environment. Assessments will consider changes in ecological function (e.g., food webs, river metabolism) and ecological traits related to factors such as life history and morphology. Finally, contaminant levels in biota and sediments will be measured, at least in part, to relate to upper trophic levels. Sampling will be multi-habitat in nature and tiered depending on priority areas or response patterns. The program will determine whether or not oil sands mining activities are having an effect on fish and benthic biota.

**475 Assessing Spatial Patterns of Polycyclic Aromatic Compounds (PACs) in the Oil Sands Region Using Passive Air Samplers** T. Harner, Environment Canada, Atmospheric Science & Technology Directorate; S. Genualdi, Environment Canada, Science And Technology Branch; J. Karpowicz, C. Mihele, C. Banic, P. Blanchard, J. Charland, Environment Canada. Development of the oil sands industry in Alberta has led to concerns regarding health risk to humans, and other terrestrial and aquatic wildlife associated with exposure to PACs, particularly along the Athabasca river and its watershed. A study was initiated in November 2010 to monitor air concentrations of PACs in the oil sands region. Passive air samplers comprising polyurethane foam (PUF) disks housed in stainless steel chambers were deployed at 17 sites, some of them close to emission sources while others at varying distances from the active zones. Compounds targeted included 16 PAHs and dibenzothiophene including their C1 to C4 alkylated homologues. The gas-phase sampling rates, sorptive capacities and resulting sample air volumes for PACs in the PUF disks were calculated based on results from previous calibration studies for nonpolar hydrophobic chemicals. For most target compounds, air sample volumes for a 1-month deployment were in the range 120 m<sup>3</sup>. For the most volatile PACs (e.g., naphthalene and alkylated naphthalenes) 'effective' air sample volumes were substantially lower due to saturation of the PUF disks (i.e., limited capacity for these compounds). The use of isotopically labeled PACs, spiked onto the PUF disks prior to deployment, is being explored as a method to improve the estimation of the sample air volumes. Air concentrations of sum of 16 PAHs in the first period samples (November 2010) ranged from 5-50 ng/m<sup>3</sup> and were dominated by naphthalene and phenanthrene. Alkylated PAHs showed greater variability among sites and ranged from 10-1000 ng/m<sup>3</sup>.

Field blanks were below detection for most target compounds. At several sites, PUF disk samplers were co-deployed alongside active air samplers which will allow for further characterization of the performance of the PUF disk sampler for gaseous and particle-associated PACs. The results from this study may also serve as a baseline against which future PAC air concentrations can be compared.

**476 Atmospheric Deposition of Inorganics and Polyaromatic Compounds in the Athabasca Oil Sands Region of Alberta, Canada** J.L. Kirk, Environment Canada, Research Scientist, Environment Canada; D. Muir, X. Wang, C. Teixeira, S. Backus, C. Mihele, G. Poole, C. Tunks, Environment Canada. Atmospheric deposition may be an important source of contaminants to the Athabasca River and tributaries in the Alberta Oil Sands (OS) region. In fact, Kelly et al., (PNAS vol 106, 2009) reported 2008 snowpack loadings of 391 kg of polycyclic aromatic compounds (PACs) equivalent to 600 T of bitumen to the Athabasca River and its tributaries within 50 km of the OS upgrading facilities. In spring 2011, we conducted similar snow surveys to Kelly et al. to quantify atmospheric loadings of unfiltered and filtered PACs, multi-elements, and mercury (Hg) to the Athabasca River and its tributaries. Snow samples were collected from 30 sites located 0-200 km from the upgrading facilities at maximum snowpack depth (late February-early March). At each site, snow pits were dug down to the bottom of level snow packs and integrated snowpack profiles were obtained using ultra-trace sampling techniques appropriate for each type of contaminant. In addition, heated wet-only precipitation samplers were installed at 3 locations within 20 km of the OS upgrading facilities and sampled monthly from December 2010 to present. Snow and precipitation were analysed for 45 elements by ICP-MS, 64 PACs by XAD resin extraction followed by GC-MS, and for total and methyl Hg by CVAFS. PAC profiles in precipitation were dominated by C1-C4 alkyl PACs, which represented 80-85% of the total PAC. Inorganics in precipitation were dominated by Al, Fe, Mn and Zn and contained ng/L concentrations of heavy metals Cd, Cu, Pb and Hg. Net spring-time loadings of contaminants to the Athabasca River and its tributaries, calculated using concentrations of contaminants in snow and average snow water equivalent, will be presented as well as an analysis of trends in contaminant deposition with respect to distance from the upgrading facilities.

**477 Assessment of Snow Melt Waters from Oil Sands Development Areas** W.P. Norwood, J. Parrott, Environment Canada, Aquatic Ecosystems Protection Research Division; P. Gillis, J. Kirk, M. Brown, C. Regan, R. McInnis, Environment Canada. *Hyalella azteca* (epi-benthic amphipod), *Lampsilis fasciola* (freshwater mussel) and *Pimephales promelas* (fathead minnow) were used to assess the toxicity of snow melt samples collected in the vicinity of the Canadian oil sands process facilities along the Athabasca River. An upstream reference site on the Athabasca River, far from the oil sands, was included in the assessment. Snow samples were collected in early March 2011 prior to spring melt and then shipped back to the laboratory frozen. Acute (24 hr) toxicity was determined with mussel glochidia (larvae) and chronic (28 day) toxicity was determined with juvenile amphipods and early life cycle tests with fathead minnows. The snow melt samples were very low in essential ions, hence they were amended with CaCl<sub>2</sub>, NaHCO<sub>3</sub>, NaBr, KCl, and MgSO<sub>4</sub> to bring major ion levels up to those observed in the Athabasca River. Amendments reversed any lethal effects on the amphipod due to low ion concentration and improved minnow survival at most sites. However, two sites were toxic to the minnows and no sites were toxic to the amphipods or mussels. An examination of contamination due to airborne pathways will be discussed. Analyses of metals, poly-aromatic hydrocarbons, naphthenic acids, major ions and standard water quality parameters will be used in the evaluation of contamination.

**478 Evaluating Human Health and Ecological Risk Assessments and Remediation Decisions: Is the Cure Worse than the Disease?** J. Toll, Windward Environmental LLC. This presentation will address the question of whether risk assessment and risk management practice should give precedence to the precautionary principle, or the Hippocratic principle "first do no harm." The purpose of the presentation is to set the theme for the rest of the session. The session is intended to provide real examples of where (and how) a risk manager balanced a conservative risk estimate against the "cons" of more stringent remedial actions in order to achieve a better outcome. We will challenge the session's presenters to consider the implications of conducting risk assessments under the premise that it's better to err on the

side of conservatism in the face of uncertainty. We will question whether this principle is appropriate for protecting human health and the environment. Does it deliver maximum benefit to local human and ecological communities? If not, then what are the opportunity costs? What if an assessment leads to an invasive remedy that damages or destroys habitat or other ecosystem assets (open space, recreation opportunities, etc.)? Is there harm associated with extensive remedies that do not yield promised reductions in health risk? Does overestimation of risk lead to faulty decision-making in selection of remedial alternatives? Are we as risk assessors and risk managers doing enough to account for the risk of remedy in our actions and decisions? Should managers focus on more than risk reduction as their performance objective?

**479 An Evaluation of Remedial Alternatives within the Context of Human Health and Ecological Risks Within an Urban Watershed** S.L. Sager, T. Schlekert, D. Malone, R. Lepsic, ARCADIS. Historical releases at a former chemical storage facility resulted in impacts to soil, groundwater, soil gas, and potentially surface water. The site is located in an urban area with mixed residential, commercial, and industrial properties. As surface water impacts were identified, Gore™ Module surveys were used to identify sources and migration pathways within the local drainage basin. The survey indicated that one of the transport pathways originates at the former storage facility while the other potentially originates from any of the multiple industrial chemical sources west of the facility. These two plumes merge and ultimately discharge to the urban surface water body. The receiving stream is rated as "Impaired" in all segments for aquatic life because the state surface water quality criterion for turbidity was violated. A variety of constituents typical of an urban environment as well as chlorinated solvents have been detected in the stream. Both a human health risk assessment (HHRA) and a screening level ecological risk assessment (SLERA) were conducted to evaluate the stream. The results of the HHRA indicated no significant risk to receptors wading in the stream while the results of the SLERA indicated a potential threat to ecological receptors. As remedial alternatives are considered, the likelihood of successful reduction in constituent concentrations is an influence on the overall strategy. If source control at the former chemical storage facility does not result in improvement in concentrations in the stream and human health is protected, then the cost of an active remediation system may not be justified given the existing impaired status of the stream for reasons unrelated to this release. This presentation will discuss the results of the risk assessments within the context of the broader implications of the need for remediation within an impaired ecosystem.

**480 Risks of Intact Residential Lead Based Paint Versus Risks of Remediation** B. Magee, A. Weaver, ARCADIS. Risk assessments often lead to decisions to take action to remediate baseline risks. Proposed remedial actions are rarely evaluated against the baseline risks to ensure that the remedy does not cause more harm than good despite the presence of Federal and State laws and regulations that generally require such risk comparisons. This presentation discusses a case study of a proposed remedial action of a massive scale and a comparative risk evaluation demonstrating that the proposed remedial action would have many negative unintended consequences. The proposal was made by a State Attorney General that all residential building components that contained lead-based paint throughout the entire State, whether intact or deteriorated, be removed or enclosed. It was proposed that all doors, windows, trim, and selected other building components be removed and replaced and that all other components, like walls, floors, and ceilings, be enclosed in place. The massive project was estimated to cost \$2.4B over four years. The Attorney General failed to consider that the mobilization of an estimated 10,000 workers to inspect homes, remove and replace building components, construct coverings over other building components, haul construction debris to landfills or incinerators, and perform all of the other elements of the proposed plan would present many health and safety risks to the workers and residents of the State. Adverse impacts discussed in this paper include: increased fatality rates due to automobile and truck traffic; increased injury rates due to automobile and truck traffic; increased cancer and respiratory injury rates due to increased vehicle emissions; increased effects on global climate change due to increased vehicle emissions; increased traffic due to delivery of construction materials and disposal of waste; increased injury rates from construction activities; increased rates of childhood and adult asthma; increased injuries to residents associated with moving personal belongings; increased stress to residents due to disruption in daily routines; increased losses of, or damage to, personal

property; increased need for temporary housing; and increased requirements for public safety services.

**481 Integrated Environmental Benefit Analysis of Sediment Remediation Thresholds** A. Morrison, Exponent, Inc., EcoSciences; S. Kane Driscoll, M.E. McArdle, Exponent, Inc.; C. Menzie, Exponent, Inc., Exponent, Principal. Decisions regarding sediment remediation must consider not only the magnitude of the risk, but also the expected effectiveness of the remedy and the potential harm of the remedy in relation to short-term and long-term effects. All of these considerations can be integrated into an environmental benefit analysis that places site-specific remedial actions within the broader context of risk reduction and remedial impact. An integrated environmental benefit analysis (IEBA) was conducted for a New England river that was impacted by metal contamination. The river was sub-divided into five reaches that are bordered by various types of habitat, including industrial and residential areas, and a large recreational park with extensive areas of aquatic vegetation. Two alternative preliminary remedial goals (PRGs) were developed based on an ecological and human health risk assessment. Both PRGs (220 mg/kg and 400 mg/kg) for sediment are fully protective of human health, wildlife, and bioaccumulation of the metal into blue crab tissue (a human health risk issue); however, the site-specific community analysis results for benthic invertebrates were ambiguous. The objective of the IEBA was to compare the benefits of dredging to remove sediments with concentrations greater than 220 mg/kg versus concentrations greater than 400 mg/kg. The percent of function, or "services", lost was calculated for each type of receptor for both remediation scenarios, and for natural recovery. These calculations estimated the percent of potentially lost services that a receptor is currently experiencing, will likely experience if current conditions prevail, or will likely experience if remediation is conducted. Estimates of percent services lost were quantitative if data were available and qualitative if data were unavailable. The area-weighted service loss for each reach of the river was combined with the estimated time to recovery for each remediation scenario. A comparative matrix of results indicated that, for most receptors and reaches of the river, outcomes under the two PRGs were comparable; however, impacts related to implementation of the 220 mg/kg PRG were much greater than the 400 mg/kg PRG in the largest reach of the river because the surface area to be dredged was substantially larger, and impacts to areas of aquatic vegetation habitat were greater. IEBA was useful in the negotiation of a remedial action plan for the river.

**483 Meeting Risk Management Goals and Minimizing Remedial Impacts for a PCB Contaminated Floodplain – The Habitat Enhancement Option** A.L. Fogg, M.H. Henning, A.R. Glessner, N.R. Pinheiro, ENVIRON International Corporation; J.M. Johnson, Bridgestone Americas, Inc. Risk assessments demonstrated that local residents and wildlife populations in an undeveloped forested floodplain of Stony Creek, located in Noblesville, Indiana (USA) are unlikely to be at risk from exposure to PCBs in floodplain soil. However, given the presence of PCB-contaminated soils with elevated concentrations in two areas of the floodplain, remedial alternatives were evaluated through a Corrective Measures Proposal (CMP). Alternatives evaluated included no action, monitored natural recovery, habitat enhancement and vegetative stabilization, capping, and focused excavation. Risk of remedy and accordance with Green Remediation practices were among the factors considered in the CMP. Based on this analysis, the US Environmental Protection Agency Region 5 selected habitat enhancement with vegetative stabilization as the corrective measure for the undeveloped floodplain. This remedy offers a net benefit to environmental conditions; is favored by the public; results in only minimal disruptions to the community during implementation; preserves the character of the land bordering residential housing; and minimizes short-term risks. Habitat enhancement involves erecting approximately 50 bat boxes to provide immediate improvement in roosting habitat for bats (including the Indiana bat) and planting 100 seedlings of tree species favored by the bat to provide long-term habitat improvement. Vegetative stabilization of floodplain soil, which involves planting five native herbaceous groundcover species, is expected to reduce soil erosion and human and ecological contact with PCB-contaminated soils in two areas with elevated concentrations. Important considerations and challenges in design, implementation, and monitoring are discussed.

**484 Guidance to Assist in the Interpretation of CCME Water Quality Guidelines for the Protection of Aquatic Life Exceedances** S. Rodney, Intrinsik Environmental Sciences, Inc.; S. Teed, Intrinsik Environmental

Science Inc., Intrinsik Environment Sciences Inc.; S. Roe, Environment Canada, National Guidelines and Standards Office; T. Fletcher, Ontario Ministry of Environment, Standards Development Branch; C. Crane, Prince Edward Island Dept of the Environment. The misapplication and misinterpretation of water quality guidelines is a significant issue in risk assessment. Guidelines are generally considered to be conservative estimates of the potential for adverse effects, but when they are incorrectly applied there can be implications to aquatic ecosystem protection, costs of remedial action, and risk management. The interpretation and significance of a water quality guideline exceedance is logically dependent on two principal factors: (1) how the guideline was derived, and (2) how the exceedance was determined (including the statistical approach, the quality of the data, the quantity of the data and how representative the data are of the water under investigation). A comprehensive review of documentation from North American and several international jurisdictions revealed that there is currently no specific guidance available on the application of water quality guidelines and the interpretation of exceedances. Most jurisdictions that derive or apply guidelines have comprehensive documentation outlining how their benchmarks are derived (e.g., CCME, USEPA, etc.). Although some of those jurisdictions provide guidance on sampling and statistics for establishing exceedances, few demand precise data requirements and/or exact statistical approaches. As such, the interpretation of benchmark exceedances is complicated by the myriad of datasets collected and computational methods that may be used to establish "exceedances". Further, ecotoxicity data are generally limited, and there are numerous other sources of uncertainty (e.g., laboratory-to-real world extrapolations). Thus, there are many sources of uncertainty around any given benchmark value for the protection of aquatic life. These uncertainties typically are unquantified and therefore interpretations of benchmark exceedances with respect to the likelihood of adverse ecological effects cannot be afforded greater confidence than the benchmark on which they were established. This guidance is intended to assist in the interpretation of exceedances of Canadian Water Quality Guidelines for the Protection of Aquatic Life. The guidance will assist environmental quality managers in determining when a benchmark exceedance is likely to, or could potentially lead to, ecologically-significant adverse effects to aquatic organisms.

**485 The Importance of Natural Recovery Rate Estimates for Application of Risk Assessment Outcomes to Remedial Decisions** J. Field, Office of Response & Restoration, NOAA, Assessment and Restoration Division, Office of Response & Restoration, NOAA, Coastal Protection and Restoration Division; J. Kern, Kern Statistical Services, Inc.; L. Rosman, Office of Response & Restoration, NOAA. Many individual assumptions in a risk assessment may be considered "conservative" without qualifying the overall risk assessment or its' application to decision-making as conservative. Temporal models are necessary to evaluate changes in exposure concentrations that would result from different remedial alternatives, including monitored natural attenuation. Without good estimates of temporal change in exposure concentrations, even using conservative risk assessment thresholds may lead to non-conservative remediation decisions. In this paper, we discuss how the overestimation of the rate of natural attenuation for sediment sites can affect discrimination among remedial alternatives and the projected time to achieve remedial action objectives. As an example, complex natural recovery models developed for the Hudson River PCBs Superfund site substantially over-estimated the rate of recovery. Surface PCB concentrations observed during remedial design sampling were higher than the upper bound of model uncertainty estimates and the concentrations following remediation are estimated to be about 5X higher than anticipated in two of three river sections, likely delaying recovery. Other sites have used approaches such as re-sampling of previously sampled locations or evaluation of dated cores to estimate rates of natural attenuation. We present recommendations for assessing temporal changes in exposure concentrations as part of the site characterization sampling plan.

**486 Advances in Ecological Modeling for Assessing Ecological Risks and Ecosystem Services** J.D. Stark, Washington State Univ, Puyallup Research and Extension Center, Dept of Entomology, Washington State Univ, Dept of Entomology. Ecosystems provide many services to human populations, including fresh water, fertile soils, pollination, climate regulation, pest control, and the decomposition of waste. Although all ecosystem services cannot be maximized all of the time everywhere – often there are tradeoffs (e.g., food production versus conservation of biodiversity), it is imperative

that we work to protect these natural assets as best as possible. Protection of the environment from human disturbance is usually accomplished through the process of ecological risk assessment. However, this process relies heavily on protection of the individual organism even though protection of populations is more important. Ecological modeling, along with standardized data packages for chemicals, can help us better protect ecosystem services by providing less uncertain risk assessments. In this talk I will provide an overview of ecosystem services and how population modeling may help improve the protection of these important assets.

**487 Modeling Ecological Effects in the USEPA** G.W. Suter, US Environmental Protection Agency, National Center for Environmental Assessment, USEPA, National Center for Environmental A; W.R. Munns, US. EPA, Atlantic Ecology Division. While simulation models are used routinely by the US Environmental Protection Agency to estimate exposure for regulatory purposes, exposure-response modeling is largely empirical. Examples include concentration-response and dose-response relationships and species sensitivity distributions. These models are used because they are familiar and easily understood, are sufficient for most routine decisions, are equivalent in human and ecological risk assessments, are supported by policy and legal precedent, and add relatively little new uncertainty to assessments and decisions. However, the Agency has developed population and ecosystem simulation models and has applied them in some cases. These models have the advantages of incorporating mechanistic relationships, being predictive of outcomes under various management scenarios, and estimating end-points that are more readily treated as benefits in economic analyses. Unlike exposure models, most effects simulation models developed by the USEPA remain research tools, with limited application in regulatory contexts. Examples include biologically-based dose response models, a variety of population dynamics models, vegetation growth models, linked Bayesian networks & community dynamics models, and energy-based models. Effects models are adopted by the Agency when they are scientifically defensible and have been demonstrated to result in better decisions. An example is the biotic ligand model which has been used to set water quality criteria.

**488 Aquatic Ecosystem Models for Risk Assessment: EPA Tools and Applications** B. Rashleigh, US Environmental Protection Agency, Research Ecologist; D.H. Miller, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; R.G. Kreis Jr, US Environmental Protection Agency. Aquatic ecosystems are subject to multiple, interacting pressures, including climate alteration, habitat alteration, toxics, and invasive species. Ecosystem modeling is a powerful tool for the assessment of risks associated with these pressures. The US Environmental Protection Agency (EPA) has developed two main models in this area. The AQUATOX model simulates the fate of pollutants, such as nutrients and organic chemicals, and their effects on the ecosystem, including fish, invertebrates, and aquatic plants. The Lake Michigan Ecosystem (LM-Eco) model has focused on assessment for the Great Lakes. LM-Eco is spatially explicit and track densities of 12 ecological compartments, including the representation of invasive mussel species. For both models, assessment of risks associated with pressures is conducted through development of baseline scenarios, calibration with field data, and comparison of alternative scenarios of impact and for restoration. Future directions for this modeling include linking aquatic ecosystem models within model frameworks to simulate future inputs and landscape changes and translate model outputs to ecosystem services and measures of sustainability.

**489 DEB-IBM, A Generic Individual-based Population Model Based on Dynamic Energy Budget Theory, Correctly Predicts Population-level Effects of Toxicants** B. Martin, Helmholtz Centre for Environmental Research, Dept of Ecological Modelling; E. Zimmer, Vrije Universiteit Amsterdam, Dept of Theoretical Biology; T.G. Preuss, RWTH Aachen Univ, Institute for Environmental Research; T. Jager, Vrije Universiteit Amsterdam; R. Nisbet, Univ of California, Santa Barbara, Ecology, Evolution & Marine Biology; V. Grimm, Helmholtz Centre for Environmental Research, Dept of Ecological Modelling. Tools are badly needed that help extrapolate the results of individual-level toxicity tests to the population level, including sub-lethal effects and different exposure scenarios. Population models can be such a tool. However, developing, parameterizing and testing such models is a major task and cannot be done from scratch for all species of interest. Therefore, population models should be based more on first principles so



that the same model structure could be used for a wide range of species. Dynamic Energy Budget (DEB) theory provides such first principles. It has been used extensively for the analysis of the lethal and sub-lethal effects of toxicants on individuals. The process-based approach used in the DEB analysis of toxicity experiments differs from standard ecotoxicological analysis (LC50, ECx, etc.) in that it includes exposure time as an explicit factor, links the effect to the internal concentration of the chemical, and explains the effects on different traits in one coherent framework. We developed a generic implementation DEB theory in an individual-based (IBM) context, DEB-IBM (<http://cream-itn.eu/projects/wp-1/daphnia-2/deb-ibm>). We parameterized DEB-IBM for *Daphnia magna*, using individual-level data on growth and reproduction, and compared population-level predictions with results from experiments. The model correctly predicted population dynamics (abundance and size structure) under control conditions. The same model correctly predicted the effect of the toxicant 3,4 dichloroaniline at the population level from data available in standard OECD tests. The advantage of developing such a model is, that when well tested, we can use it to understand how varying exposure scenarios or environmental conditions, would influence the population-level response to a toxicant. Lastly, because DEB can be used to model, in principle, all animal species, we believe DEB-IBM has great potential for population-level risk assessment because the same general model structure can be used for different test organisms.

#### 490 Ecological Models in Risk Assessment – A Regulatory Perspective

M. Reed, Chemicals Regulation Directorate. Ecological models allow environmental risk assessments account for effects on populations rather than single species in laboratories. A variety of models have been developed, particularly for aquatic organisms, birds and mammals, but their use in regulatory risk assessments has so far been limited. One of the benefits of ecological models is they can be used to link the output from toxicity tests and predictions of exposure directly to protection goals. Ecological models are routinely used in other areas of ecosystem decision making, e.g., fisheries management. The use of the ecosystem services approach has highlighted that for most groups the entity that needs to be protected is the (meta) population, although for vertebrates the individual may be important. In this context, ecological models are particularly useful for aquatic and terrestrial invertebrates to assess the impact on populations where either acute or chronic effects are predicted from laboratory data. For vertebrates they are useful where the chronic effects are predicted and a more ecologically realistic assessment is required. When considering an ecological model for risk assessment a regulator will have a number of questions in their mind: Is the model appropriate? Has it been validated? Are the input parameters justified? What are the critical parameters? What does the output mean? How does it relate to the protection goals? These areas need to be addressed. Any modeling approach also needs to be fitted within the context of the overall risk assessment. Where models are submitted for regulatory use it is important for the regulator to have confidence that the model is sensitive enough to detect adverse effects that may occur in real ecosystems in the same way that a toxic standard is used to test the sensitivity of laboratory and field systems. Another issue that needs to be addressed is whether a suite of standard models (similar to the routine use in exposure modeling) is needed or whether individual models should be produced to answer specific questions. Ecological modeling (via standard models) has been highlighted as necessary to address the specific protection goals currently being developed. In other areas the questions to be resolved are pesticide specific, so there will be a need for bespoke models. Although experience of evaluating ecological models for pesticide registration has so far been limited important lessons have been learnt about how models should be presented and applied.

#### 491 Including Ecosystem Services in Risk Assessments: Using Ecological Modelling to Link Ecotoxicological Data with Protection Goals

P. Thorbek, Syngenta Ltd, Environmental Safety, Syngenta, Environmental Safety; P. Edwards, R. Murfitt, D. Huggett, Y. Bramley, E. Farrelly, Syngenta Ltd, Environmental Safety; R. Brain, P. Hendley, Syngenta, Environmental Safety. The European Food Safety Authority (EFSA) recently published an opinion regarding environmental protection goals which was based on the ecosystems services concept. The opinion stated that, for most ecosystem services, the protection goal is at the population level of organisation (although for vertebrates the protection goals may remain at the individual level for cultural reasons). This is in contrast to current standard ecotoxicity data requirements for pesticide registration that are focused at the level of individuals. However, ecological modelling can make use of standard

data to assess the potential risks that exposure to pesticides may present to populations or higher levels of organisation and is compatible with current regulation. Here we propose a tiered system for how ecological modelling can bridge the gap between data at the level of individuals and the protection goals. We then present examples of how different types of ecological modelling can be used to refine ecological risk assessments for different pesticides. The first example demonstrates how a simple, worst case, toxicokinetic model based on field bird feeding rates can help refine avian risk assessment. The second example shows how an individual-based population model was used to perform a population level risk assessment for invertebrates. Finally we demonstrate how ecosystem models can be used to assess the risk of indirect effects on aquatic ecosystems. These examples demonstrate how ecological models can help refining risk assessments and extend the use of standard data to increase the relevance of risk assessments for protection goals.

#### 492 Internal Dose Metrics and Toxicokinetic Modeling as the Link between Intentional Dosing and Environmental Exposure

W. Schmitt, Bayer CropScience AG, Bayer CropScience AG, Environmental Modelling. A challenge frequently arising in ecological risk assessments for xenobiotics is the extrapolation from exposure patterns, typically applied in toxicity tests, to realistic exposures as they occur in nature. Basing the risk assessment on internal concentrations, i.e., the body burden, instead of external exposure leads to a solution for this issue. For the translation between external and internal exposure toxicokinetic (TK) models are needed that take into account the relevant fate processes as absorption, metabolism and excretion as well as species specific behavior like feeding patterns and avoidance. The methodology of TK modeling is in principle applicable to all kinds of biota and thus also in terrestrial as well as aquatic risk assessments. The complexity of the models, however, may vary strongly depending on the species they are developed for and the specific situations to be dealt with. In aquatic risk assessments for pesticides it is an often occurring situation that the exposure in water bodies close to treated fields is very transient, while the toxicity is determined under more or less static exposure conditions. For the example of aquatic macrophytes it will be discussed how the consideration of TK applying a suitable model allows to estimate effects of growth inhibiting substances under realistic conditions based on standard test results. A further example will be given on the consideration of feeding patterns of small mammals and birds for the estimation of body burdens in risk assessments. For acute toxicity tests dosing is often done by gavage leading to high peaks in internal concentration while under ecological conditions food uptake is usually distributed over a longer period leading to lower, but potentially longer lasting body burdens. For this purpose a physiologically based toxicokinetic (PBTK) model was developed that allows the consideration of a toxic metabolite in addition to the parent compound.

#### 493 Modelling Honey Bees: Colony Dynamics, Foraging and Parasites

M.A. Becher, Rothamsted Research, Plant and Invertebrate Ecology; V. Grimm, Helmholtz Center for Environmental Research – UFZ; P.J. Kennedy, J.K. Pell, Rothamsted Research, Plant and Invertebrate Ecology; D. Chandler, Univ of Warwick; P. Thorbek, Syngenta Ltd., Jeallott's Hill; J.L. Osborne, Rothamsted Research. Substantial losses of honey bee colonies have been reported in recent years. It is believed, that no single factor is responsible for the colonies decline but the interaction of several stressors weaken the colonies until they collapse. Important factors are parasites and diseases, availability of forage and beekeeping practices. To understand the interaction of these factors we have developed a computer model, simulating the dynamics of a single honey bee colony. In order to keep computational time low but to allow for flexibility in the decision-making we combined a cohort based population model with an agent based foraging module. We included the Varroa mite as a bee parasite which acts as vector for two different bee viruses, the deformed wing virus and the acute paralysis virus. Crop maps defining the availability of food sources can be used in a separate, spatially explicit landscape model to allow the application of realistic foraging scenarios with specifically defined nectar flows over time. Exposure of pesticides might be included in a further step. For validation of the model we will take advantage of the considerable datasets already existing (e.g., COLOSS, German Bee Monitoring Project). In this talk we will describe and explain the model's design and how it relates to existing honey bee models. We will then present verifications of the submodels and first results of the full model where in hypothetical scenarios the relative importance of the stressors is

analyzed. Finally, we will outline further steps to be taken with our model and how it could be used for better understanding the decline of honey bees.

**494 Critical Radionuclide/Critical Pathway Analysis for the US Dept of Energy's Savannah River Site** G.T. Jannik, Savannah River national Laboratory, Environmental Analysis; W. Kuhne, Savannah River national Laboratory, Environmental Analysis, Medical College of Georgia, Institute of Molecular Medicine and Genetics. Many different radionuclides were released to the environment from the Savannah River Site (SRS) during its operational history (1954 – present). However, only a small number of the released radionuclides were relatively significant contributors (via a small number of critical pathways) to potential doses and risks to offsite people. This paper presents the results of the radiological critical-contaminant/critical-pathway analysis currently being performed for SRS. The major steps in performing public radiation dose and risk assessments are (1) characterization and quantification of source terms, (2) calculation of atmospheric and surface water transport (dispersion/dilution), (3) identification and quantification of environmental pathway transport to humans (exposure pathways), and (4) calculation of radiation dose and subsequent risk. The analysis covers radiological releases to the atmosphere and to surface water, which are the principal media that carry contaminants off site. The analysis also incorporates the recent findings of the SRS Performance Assessment and Composite Analysis Programs. In keeping with the theme of this Environmental Radiation Special Session, a concluding discussion of the major uncertainties in the analysis and "what should we know for assessing risks" is provided.

**495 Addressing Concerns About Wild Foods Safety in Alaska Following Radiation Releases in Japan** L. Verbrugge, US Fish and Wildlife Service, Anchorage Fish and Wildlife Field Office; L. Castrodale, State of Alaska, Dept of Health and Social Services, Division of Public Health, Section of Epidemiology; R. Klein, Alaska Dept of Environmental Conservation, Division of Environmental Health. Many Alaskans rely heavily on wild foods for subsistence, especially Alaska Natives and others that live in remote rural areas with no road access. Following the March 2011 radiation releases from the Fukushima Daiichi nuclear power plant in Japan, state and federal agencies in Alaska received questions from the public about whether radiation was making wild foods unsafe to eat. An inter-agency workgroup with expertise in health, wildlife, and the environment was formed to evaluate the public's concerns and to provide information. Air monitoring revealed that radiation levels reaching Alaska through the air were very small, and the US Food and Drug Administration provided helpful information about the safety of the commercial food supply in the United States. Alaska's workgroup carefully evaluated the potential risk posed by birds that overwinter in or migrate through Japan on their way to breeding grounds in Alaska. Wildlife agencies compiled a list of migratory birds in Alaska categorized by migration pattern/risk of exposure, environmental toxicologists evaluated scientific literature to assess the fate and behavior of several important radionuclides in the environment, and health officials assessed potential health risks and provided health-related advice. The interagency team worked together to develop joint information materials that were posted to the Internet. The team found that the likelihood of encountering a bird with dangerous levels of radiation in Alaska while subsistence hunting is very slight, for a number of technical reasons that will be presented.

**497 Modeling Radionuclide Bioaccumulation in Aquatic Foodwebs that Receive Episodic or Pulse Releases: Steady State or Biokinetic?** D.J. Rowan, Atomic Energy of Canada Ltd., Environmental Technologies Branch. Episodic or pulse releases of radionuclides and other contaminants to aquatic ecosystems are common, with releases from the damaged reactors at Fukushima an extreme example. However, the effects of episodic releases on bioaccumulation remain poorly understood. This likely stems from the lack of data on contaminant concentrations in water and biota prior to a release, during a release and after a release. Without prior knowledge of the timing of a pulse release, an ongoing, detailed time series study of contaminant concentrations in an aquatic food web would have to serendipitously straddle a pulse release and include its contaminant(s). While conducting a detailed time series study of cesium isotopes in a pelagic food web, several small pulse releases of Cs-137 occurred, yielding an unusual data set that consists of two isotopes of the same element under steady state (stable cesium, Cs-133) and non-steady state (radiocesium, Cs-137) conditions. Cesium enters aquatic food-webs through direct uptake by primary producers, followed by trophic transfer to higher trophic levels. This provides

a unique opportunity to compare the performance of steady state and biokinetic approaches. Stable cesium was used to calculate bioaccumulation factors (BAFs) for weekly to bi-weekly collections of zooplankton, smelt (age-class composites) and walleye (individual fish) and to parameterize a biokinetics model. I compare the predictions of steady state and biokinetic models with respect to observed Cs-137 concentrations in fish over the eight month study period. The biokinetic, mechanistic food web model predicts a range of Cs-137 concentrations that encompasses observed concentrations in Ottawa River pygmy smelt (*Osmerus spectrum*) and walleye (*Sander vitreus*), describes temporal variation due to pulse releases and handles the complexity of a partially mixed source term. The steady state approach exhibits poor predictive capability during the entire eight month study period even though the BAFs used were not generic, but were derived from the same samples.

**498 Modeling the Bioaccumulation Potential of Cesium 137 in a Marine Food Web of the Northwest Pacific, Canada** J.J. Alava, Simon Fraser Univ, Resource & Environmental Management; E.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment), Simon Fraser Univ. The Fukushima nuclear emergency provoked by the tsunami that impacted the north east coast of Japan on March 11, 2011, emerged as a high priority looming threat due to the risk of radioactive contamination in the global ocean and biodiversity. On April 11, The Fukushima nuclear plant reached the severity level 7, the same as the 1986 Chernobyl nuclear disaster. One of the most persistent isotopes produced and expected to be released by this kind of nuclear accidents was Cesium 137 ( $^{137}\text{Cs}$ ), with a physical half life of 30 years. In an effort to assess the fate, accumulation and health effects of  $^{137}\text{Cs}$  in marine organisms of the Northwest Pacific after the Fukushima nuclear disaster, we assessed the bioaccumulation potential of  $^{137}\text{Cs}$  by testing steady state and time-dependent bioaccumulation models in an offshore food web that included fish-eating, resident killer whales (*Orcinus orca*) as one of the major top predators of the marine ecosystems in British Columbia, Canada. The steady stated model showed that concentrations of  $^{137}\text{Cs}$  predicted in the male killer whale were approximately three orders of magnitude higher relative to its major prey, Chinook salmon, and > 13,000 times higher compared to phytoplankton. The time-dependent model showed that after 30 days of radioactive spillage, the  $^{137}\text{Cs}$  concentrations accumulate gradually over time in high trophic level organisms (salmon and killer whales), which exhibited low concentrations likely driven by slow intake rates, while it bioaccumulates at faster uptake rates in low trophic level, gill ventilating organisms (phytoplankton, zooplankton, benthic invertebrates and planktivorous fish), exhibiting concentration about one to two orders of magnitude greater than that in killer whales. At 9125 days (25 years), the predicted concentrations of  $^{137}\text{Cs}$  accumulate in a higher degree in killer whales, being >2 orders of magnitude greater than that predicted in Chinook salmon and 10,000 times higher relative to phytoplankton. The levels of  $^{137}\text{Cs}$  predicted in biota (shellfish and fish) exceeded well above the  $^{137}\text{Cs}$  action level for commercial food/beverage of 1000 Bq/kg established by the Canadian Guidelines for Consumption following a Nuclear Emergency.

**500 The Only Certainty is Uncertainty: Parameter, Model, and Scenario Uncertainties in Radioecology** L. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center; F. Carini, Universita Cattolica; C. Lu, MIT. Modelling is widely used to predict the fate of radionuclides in ecosystems following accidental releases. Understanding uncertainty associated with modelling is therefore an important aspect not only of conducting analysis consistent with current regulatory guidance, but also of gaining stakeholder and decision-maker trust in the process and confidence in the results. In the framework of the BIOSphere Modeling and ASSESSment (BIOMASS) program, the International Atomic Energy Agency (IAEA) developed several scenarios that described specific cases of radionuclide releases, and then tasked individual modelers or groups of modelers with the prediction of contaminant fate and transport in ecosystems. The BIOMASS Fruits Working Group undertook modelling studies to identify and investigate significant areas of uncertainty in modelling radionuclide transfer to fruit, with particular attention to "modeller uncertainty," i.e., differences in problem formulation, model implementation and parameter selection originating from subjective interpretation of the problem at hand. Two different exercises were carried out: model-model intercomparisons, where only the differences among model predictions were evaluated, and model-data

intercomparisons, where model predictions were compared to measured field data. This presentation will summarize Parameter, Model and Scenario uncertainty resulting from multiple modelling exercises from the BIOMASS framework. The results show that even for the simple and well-defined scenario of radionuclide accumulation in agricultural fruits (relative to more complex ecosystems), the differences in model predictions may be as high as five orders of magnitude for short term predictions following the acute radionuclide deposition. Comparisons with other fields and authors perspectives on modelling for regulatory risk assessment will be discussed.

**501 Why We Still Need Data for Radioecological Modeling** K. Higley, Oregon State Univ, Nuclear Engineering and Radiation Health Physics; D. Bytwerk, E. Houser, Oregon State Univ. Radioecologists are damned by their own apparent success in the proliferation of tools for assessment of dose from radiological releases. In addition to human exposure and dose assessment is an area that is still evolving: radiological dose assessment for nonhuman biota. Until recently dose calculation to species other than humans were regarded as unnecessary in radiological assessments. Transport and exposure pathways were evaluated predominantly for biota present in the human food chain. Changes in philosophy regarding protection of the environment, as well as the need to be more comprehensive in assessing impact have forced a reconsideration of this approach. However, biota dose assessment is in its infancy, and made more difficult because of the complex nature of food webs in nature. The requirement to make predictions of radionuclide transfer at many trophic levels in an ecosystem in order to fully assess uptake and exposure of a particular species is formidable. Dose assessment of non human biota is still evolving, and the tools are still being developed and debated. Two recent events have underscored the importance of data collection for radioecological modeling for both humans and biota. First, the publication by the International Atomic Energy Agency (IAEA) of two documents – IAEA TECDOC 1616 (2009) “Quantification of Radionuclide Transfer in Terrestrial and Freshwater Environments for Radiological Assessments, and Technical Reports Series No. 472 (2010) “Handbook of parameter values for the prediction of radionuclide transfer in terrestrial and freshwater environments” illustrate that with the exception of a few very well studied radionuclides such as  $^{137}\text{Cs}$ ,  $^{90}\text{Sr}$ , and  $^{131}\text{I}$  there are substantial data gaps in our knowledge of environmental transfer of radionuclides. Secondly, the events resulting from the nuclear plants in Fukushima Daiichi Japan highlight our need to quickly and accurately forecast near term environmental behavior of radionuclides so as to help limit both radiological and economic impacts. Specifically, increased accuracy in our models could help better delineate “embargo” zones for the purpose of restricting agricultural activities to limit consumption of contaminated foodstuffs and to provide some degree of certainty to farmers. Such efforts need to be made quickly and as precisely as possible – particularly in circumstances such as in Fukushima where the releases were in advance of the growing season for many crops.

**502 Arsenic Toxicity to Juvenile Fish: Effects of Exposure Route, Arsenic Speciation, and Fish Species** R.J. Erickson, USEPA, Mid-Continent Ecology Division; D.R. Mount, US Environmental Protection Agency, ORD; J. Fernandez, USEPA, Research Chemist; T. Highland, J.R. Hockett, USEPA, Mid-Continent Ecology Division; D. Hoff, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; C. Jenson, USEPA, Mid-Continent Ecology Division. Arsenic toxicity to juvenile rainbow trout and fathead minnow was evaluated in 28-day tests using both dietborne and waterborne exposures, both inorganic and organic arsenic species, and both a live diet and an arsenic-spiked pellet diet. Effects of inorganic arsenic on rainbow trout growth were elicited when dietary concentrations were  $>10\text{ }\mu\text{g As/g dwt}$ , but no effects of dimethylarsinate, monomethylarsonate, and arsenobetaine on growth were observed at dietary concentrations at least 10-fold higher. Exposure of the live diet organisms to either arsenite or arsenate resulted in similar relationships of fish growth to dietary concentration, because arsenate was reduced to arsenite in the diet. For arsenite, dietborne exposure growth effects were similar whether a live or pellet diet was used. Waterborne exposures of rainbow trout to  $\geq 16\text{ mg As/L}$  of arsenate resulted in both growth reductions and mortality, but these water concentrations were much greater than those needed to contaminate live diet organisms enough to elicit growth effects. When growth effects were based on accumulation in the fish, waterborne and dietborne exposures showed similar dosimetry and were roughly additive. Fathead minnows were

more sensitive than trout to mortality from waterborne exposure, but less sensitive to growth effects from dietborne exposure.

**503 Effect of Allometry on Predicted Steady State Metal Accumulation** P. Paquin, HDR|HydroQual; R. Mathew, Moffatt & Nichol; R. Santore, HDR|HydroQual. Kinetic models are frequently used to predict the bioaccumulation of trace elements in aquatic organisms. These models are commonly applied in steady state mode to evaluate tissue residue concentrations, bioconcentration factors (BCFs), bioaccumulation factors (BAFs), and the like. The approach is based on an underlying assumption that the kinetic coefficients used in the evaluation remain constant over time. In contrast to this assumption, it has frequently been shown that several important model coefficients conform to allometric relationships. As such, kinetic coefficients such as those related to metal uptake rate from water, metal assimilation efficiency from food, ingestion rate of food and growth rate may vary with organism size. The result is that the presumably constant values for such coefficients are actually transient in nature. That is, they will change over time as an organism grows. It has also been found that BAFs observed in nature can vary with the metal concentration in water. It is for these reasons that the predicted BAFs and steady state tissue concentrations are likely to be somewhat illusory under real world conditions. A time variable bioaccumulation model that was developed for bivalves will be used to assess the importance of the preceding considerations when predicting steady state tissue residue levels for copper.

**504 Biologically-mediated Flux of Trace Metals from Streams to Riparian Spiders: A Large Scale Survey in Mineralized Alpine Ecosystems** J.M. Kraus, T. Schmidt, D. Walters, R. Wanty, R. Zuellig, C. Stricker, P. Lamothe, US Geological Survey. Trace-metal contamination of aquatic ecosystems is a major health and environmental concern globally. In the Rocky Mountains, geologic processes and mining can strongly alter the distribution and bioavailability of metals within streams, reducing aquatic insect production ( $\text{g C/m}^2/\text{yr}$ ) and increasing metal body burdens. Here we use a large scale (4,488  $\text{km}^2$ , ~30 streams) survey to examine whether these effects on stream macroinvertebrates can cascade across ecosystem boundaries to alter neighboring terrestrial ecosystems in the Central Colorado Mineral Belt. We ask how bioavailable metals in stream alter metal concentrations and emergence production of aquatic insects, and the metal body burdens and biomass of riparian web-building spiders (Tetragnathidae). Because aquatic insects emerge from streams as adults, metals bioaccumulated in their bodies may negatively affect terrestrial spiders by reducing the biomass of available aquatic prey and by increasing their metal exposure. Results presented will highlight how geology, human disturbance, and ecological responses of stream and riparian biota determine fluxes of aquatically derived metals to terrestrial food webs. This study quantifies transport mechanisms and potential effects of aqueous metals on terrestrial ecosystems to better understand direct/indirect exposure and risks to riparian consumers posed by upstream mining activity. Implications for stream and riparian management in the national forests and parks where this study takes place are discussed.

**505 Cadmium, Zinc, Manganese and Calcium Transport in a Stream Insect (*Hydropsyche sparna*): Interactions, Anomalies and Adsorption** M.D. Poteat, North Carolina State Univ, Dept of Environmental and Molecular Toxicology; M. DiazJaramillo, Universidad de Concepcion; D.B. Buchwalter, North Carolina State Univ, Dept of Environmental and Molecular Toxicology. Despite their ecological dominance and prevalent use as ecological indicators, the trace element physiology of aquatic insects remains poorly studied. While work with fish and crustaceans strongly suggests that Cd and Zn are transported apically through Ca-related transport systems, few studies with insects are available for comparison. Ca is largely thought to afford protection against Cd and Zn uptake and toxicity. Mn, a Ca analog, could also conceivably offer protection via competition for uptake or via the formation of oxide phases, which are known to be sinks for other metals. Here we used a radiotracer approach to examine interactions among Cd, Zn, Ca, and Mn. At environmentally relevant concentrations (2.7 nM Cd and 45.9 nM Zn), Cd and Zn did not compete for uptake, suggesting ample transport capacity consistent with a calcium or bulk cationic transport system. At extreme concentrations (8.9  $\mu\text{M}$  Cd and 15.3  $\mu\text{M}$  Zn), Cd accumulation was unaffected by the presence of Zn whereas Zn accumulation rates were 58% slower in the presence of Cd. Surprisingly, accumulation rates of Cd and Zn were remarkably similar under low Ca (31  $\mu\text{M}$ ) and high Ca (1.3 mM) concentrations. External Mn concentrations (up to 18.2  $\mu\text{M}$ )



decreased adsorption of Cd and Zn in a concentration dependent manner, but did not alter absorbed concentrations of these elements. Comparisons of rinsing protocols to examine metal adsorption profiles revealed an association of Cd and Zn with oxide phases on the insect body surface. The addition of a 0.1 M ascorbate rinse to a 0.05 M EDTA rinse typically removed 18.5±8.7% more Cd and 13.8±4.7% more Zn than the EDTA rinse alone. Pharmacological blockers of Ca transport were used to examine Ca, Cd, and Zn uptake routes. L-type Ca channel blockers verapamil and nifedipine along with plasma membrane Ca-ATPase inhibitor carboxyeosin had no influence on Ca, Cd, or Zn uptake rates at concentrations up to 100 µM. Ruthenium red, a Ca-ATPase inhibitor, significantly decreased Ca absorption by 53% at 10 µM and by 93% at 100 µM. At 100 µM, ruthenium red significantly reduced total Zn accumulation rates and final internalized concentrations by 89% and 80%, respectively, and reduced total Cd accumulation rates by 87% and internalized concentrations by 71%. These results suggest that Zn and Cd do traverse a Ca-ATPase transport system, but that ambient Ca only exerts minor influence in Cd and Zn uptake.

**506 Chronic Nickel Bioaccumulation, Sub-cellular Fractionation and Essential Ion Disruption in Two Teleosts: The Round Goby and the Rainbow Trout** E.M. Leonard, U. Banerjee, J. D'Silva, C.M. Wood, McMaster Univ, Biology. The Tissue Residue Approach (TRA) is a relatively new concept that predicts toxicity as a function of metal levels within the organism at an organ or cellular level. The aim of this model is to determine a range of tissue residues that cause harmful effects on organisms due to multiple routes of entry (i.e., waterborne and/or dietary exposures). Therefore, round gobies (*Neogobius melanostomus*) and rainbow trout (*Oncorhynchus mykiss*) were exposed to a joint waterborne and dietary nickel (Ni) exposure for 30 days and were sampled on days 0, 2, 4, 10, 20, 30 for whole-organ bioaccumulation, sub-cellular fractionation and essential ion homeostasis. Sub-cellular fractionation of the individual organs was conducted to assess internal Ni processing and distribution within the cells. Ni bound to the organelles and heat-denaturable proteins are considered the Metal Sensitive Fraction (MSF) and Ni bound to the metal rich granules and metallothionein like-proteins are considered detoxified and part of the Biologically Detoxified Metal (BDM). Round gobies were approximately two times more sensitive than rainbow trout to a 30-day Ni exposure. There was no effect of Ni on growth in either teleost. The general trend of Ni accumulation was the gills>kidney>gut>liver. In the gills there was an initial increase in Ni burden followed by a recovery back to control levels by day 30 in both species. Ni bioaccumulation in kidney tissue increased over time in both species. In round gobies, this significant increase was correlated to the highest mortality at 50 µmol Ni/L. Sub-cellular fractionation of round goby gills showed significant Ni accumulation in the organelles over time which caused a ~13% increase of Ni in the MSF and a ~10% decrease of Ni in the BDM. Overall, mortality in the round goby may be related to Ni bioaccumulation in the kidney and an increase of Ni in the MSF of the gills. In gobies, acute Ni exposure led to a 50-80%, ~60% and ~58% decrease in gill Ca, Mg and Na levels, respectively. Gill essential ion concentrations returned to control values by day 30. Kidney ion homeostasis was maintained over the 30 day exposure. Additionally, a comparison between rainbow trout and round gobies will be discussed in terms of Ni bioaccumulation, sub-cellular analysis and essential ion disruption in the liver and gut. (NSERC Strategic Grant, Rio Tinto Alcan and Environment Canada).

**507 Copper Uptake by Sodium Transporters and its Distribution in the Blue Crab *Callinectes sapidus* Acclimated to Low Salinity** C.d. Martins, M.B. Jorge, M.M. Giacomini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas; C. Wood, McMaster Univ, Dept of Biology; A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. In vivo and in vitro studies were performed to evaluate copper flux through sodium transporters and its organ-specific distribution in the blue crab *Callinectes sapidus* acclimated to low salinity (2 ppt). At low salinity, the blue crab hyperosmoregulates with a major ion uptake. For the in vivo tests, adult crabs were exposed (6h) to 1 µM of <sup>64</sup>Cu in artificial seawater and in a sodium free media, both at 2 ppt, with the following apical blockers: amiloride (100 µM) and furosemide (120 µM). For the in vivo tests, isolated gills were perfused (4h) with a saline solution corresponding to hemolymph and incubated with a saline solution corresponding to seawater at 2 ppt with amiloride or furosemide and 1 µM of <sup>64</sup>Cu. Samples of the incubation media and hemolymph (in vivo) or perfusate (in vitro) were collected over the respective experimental period.

The efficiency of amiloride and furosemide in blocking sodium uptake in vivo and in vitro was first checked by adding <sup>24</sup>Na in the exposure media. Results from sodium flux in vivo and in vitro showed that both amiloride and furosemide, in less extent, are able to block sodium uptake. Copper uptake from water in both artificial seawater and sodium free media did not change with presence of blockers, at a rate of approximately 3.0x10<sup>-4</sup> µmol.g<sup>-1</sup>.h<sup>-1</sup> in all treatments. In vitro experiments with isolated-perfused gills also showed that amiloride and furosemide are not able to block copper influx. Concerning copper organ-specific distribution, in vivo tests showed that <sup>64</sup>Cu was primarily accumulated in the gills, exoskeleton and carapace rather than in the hemolymph, hepatopancreas, or other internal tissues. According to the presented findings, copper seems to be incorporated by blue crabs through other transporters than those responsible for sodium uptake. Alternatively, it is possible that copper uptake occurs essentially via specific copper transporters in *C. sapidus* since a significant amount of this metal is required for the respiratory pigment of crustaceans, hemocyanin. Moreover, our results indicate that gills are a key target organ for copper accumulation and that following its entry into the hemolymph it is distributed to organs where it will not be so harmful. This phenomenon may be a detoxification response as copper accumulated in carapace can be lost during molting. (Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), International Development Research Centre (IDRC))

**508 Food Rationing Affects Dietary Selenium Bioaccumulation and Life Cycle Performance in the Mayfly *Centroptilum triangulifer*** J.M. Conley, North Carolina State Univ, Dept of Environmental and Molecular Toxicology; D.H. Funk, Stroud Water Research Center; D.B. Buchwalter, North Carolina State Univ, Dept of Environmental and Molecular Toxicology. Selenium effects in nature are mediated by the relatively large bioconcentration of aqueous Se by primary producers and smaller, yet critical, dietary transfers to primary consumers. These basal processes are then propagated through food webs to higher trophic levels. Here we quantified the movement of dissolved Se (as selenite) to periphyton, and used the resultant periphyton as a food source for conducting full life-cycle dietary Se exposures to the mayfly *Centroptilum triangulifer*. Periphyton bioconcentrated Se ~2200-fold from solution in a log-linear fashion over dissolved Se concentrations ranging from 1.1 to 23.1 µg L<sup>-1</sup>. We examined the influence of two feeding ration levels (1x and 2x) on trophic transfer, tissue Se concentrations, maternal transfer, and functional endpoints of mayfly performance. Mayflies fed a lesser ration (1x) displayed greater trophic transfer factors (mean TTF, 2.8 ± 0.4) than mayflies fed 2x rations (mean TTF, 1.1 ± 0.3). In 1x exposures, mayflies exhibited significant (p < 0.05) reductions in survivorship and total body mass at dietary [Se] ≥ 11.9 µg g<sup>-1</sup>, reduced total fecundity at ≥ 4.2 µg g<sup>-1</sup>, and delayed development at ≥ 27.2 µg g<sup>-1</sup>. Mayflies fed a greater ration (2x) displayed reduced tissue Se concentrations (apparently via growth dilution) relative to 1x mayflies, with no significant effects on performance. These results suggest that the influence of Se on mayfly performance in nature may be tied to food resource availability and quality. Furthermore, nutritional status is an important consideration when applying laboratory derived estimates of toxicity to risk assessments for wild populations.

**509 Uptake of Platinum Group Elements by the Marine Phytoplankton *Chlorella stigmatophora*, Their Fate and Effects** L. Shams, Univ of Plymouth, School of Geography, Earth and Environmental Sciences, Univ of Plymouth, School of Geography, Earth and Environmental Sciences; A. Turner, M. Brown, G. Millward, Univ of Plymouth. Very little information exists on the marine biogeochemistry of Rh, Pd and Pt, or the platinum group elements (PGE), an emerging group of contaminants whose principal emissions are associated with the abrasion of the catalytic converter in motor vehicles and chemotherapy drugs discharged in hospital wastes. In this study, PGE were added individually and in combination to cultures of the marine microalga, *Chlorella stigmatophora*, and the accumulation of metal was established under varying conditions (pH, algal biomass, PGE concentration, time). Under all conditions the extent of accumulation was in the order: Rh > Pd >> Pt. In short-term (24-h) exposures, accumulation isotherms were quasi-linear up to PGE concentrations of 30 mg L<sup>-1</sup>, although Pd displayed convexity, hence saturation of available binding sites, at greater concentrations. The pH did not have a great impact on PGE accumulation, with Rh displaying a moderate increase in accumulation and Pd a moderate reduction with increasing pH. More important, all PGE displayed a significant reduction in accumulation on a weight-normalized basis with

increasing concentration of algae, an effect not reported for metal-marine algal interactions previously in the literature. Longer-term experiments showed that the rates of both overall accumulation and internalization were greatest for Pd and least for Pt. Consistent with this observation, the toxicity to *C. stigmatophora* evaluated by both pigment content and growth rate, was significantly greater for Pd. Differences in the biogeochemical behaviours among the PGE are attributed to differences in their aqueous speciation in seawater, different affinities for the algal surface, different tendencies to cross the cell membrane, and especially with regard to Pd and Pt, differences in the rates of these interactions. Because the environmental concentrations of PGE are predicted to increase with increasing emissions from vehicles and hospitals, the results of this study make an important contribution to an improved understanding of the likely effects and fates of these emerging contaminants in the marine environment. The results are also more generally important to an improved understanding of the interactions of trace metals with microalgae in seawater.

**510 Soil Ingestion as a Pathway of Exposure to Lead in Ground-feeding Songbirds** W.N. Beyer, US Geological Survey, Patuxent Wildlife Research Center; B. Spears, US Fish and Wildlife Service, Northern Idaho Field Office; J. Hansen, US Dept of Energy, Richland Operations Office. Documented lead poisoning of birds has generally been the result of ingestion of lead shot or sinkers. In mining areas such as the Coeur d'Alene River Basin in Idaho, however, waterfowl have been poisoned from incidental ingestion of lead in contaminated sediments. We investigated soil ingestion as a potential pathway of exposure to 205 ground-feeding songbirds at the same site. Based on the aluminum content measured in ingesta of these birds, we estimated surprisingly high rates of soil ingestion, about 20% in American robins (*Turdus migratorius*) and 17% in song sparrows (*Melospiza melodia*). Almost all of the lead ingested by these species was associated with soil rather than the soil-free diet. Swainson's thrushes (*Catharus ustulatus*), which feed higher than the two other species, ingested an estimated 1% soil in their diets. Based on activity of the enzyme ALAD and on analyses for lead in blood and livers, we concluded that many of the robins and song sparrows from the most contaminated sites were exposed to toxic concentrations of lead. Species of songbirds not normally feeding on the ground would be expected to have low exposures to lead. Assessments on wildlife exposed to lead should include soil or sediment ingestion as a pathway of exposure, rather than rely solely on biomagnification estimates.

**511 What Can We Learn About Exposure and Effects of Rodenticides to Nontarget Wildlife from Investigations Following Broad Scale Conservation Use?** N. Golden, US Fish and Wildlife Service, Environmental Contaminants Program; P. Dunlevy, USDA/APHIS Wildlife Services; S. Ebbert, US Fish and Wildlife Service, Alaska Maritime National Wildlife Refuge; C. Swift, US Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office. The frequency of anticoagulant rodenticide detection in wildlife has led the US Environmental Protection Agency to impose restrictions on the sale and use of these pesticides aimed at reducing exposure to nontarget organisms. While the curbing of residential and commercial use is being pursued, anticoagulant rodenticides, particularly diphacinone and brodifacoum, remain a common tool of government agencies and non-governmental organizations in the US for the control of invasive species for conservation purposes. In particular, eradication of invasive rodents from islands has become a high-profile conservation tool for habitat restoration, and rodents have been eradicated from hundreds of islands worldwide using rodenticides. In order to expose all target individuals to lethal doses, rodenticides are often applied to these islands at rates that are much higher than have been used in commensal or agricultural settings, and exposure to nontarget organisms present in and around the application area is nearly impossible to avoid. As such, these applications can serve as a means to investigate exposure pathways and adverse effects associated with these rodenticides that would be difficult to gather from other registered uses, including pathways that are not always predicted based upon our prior knowledge of rodenticide properties or animal behavior. Data generated from recent post-eradication monitoring and investigations of nontarget mortality reveal new information about diphacinone and brodifacoum, that when combined with laboratory studies, can influence not only the development of future eradication efforts, but potentially risk assessments for other uses of anticoagulant rodenticides. Consolidation of this data and identification of data gaps can also serve to guide the prioritization of areas for further investigation.

**512 Mercury in Waterfowl from a Contaminated River in Virginia** D.A. Cristol, College of William & Mary, Professor of Biology, College of William & Mary, Dept of Biology; L. Savoy, D. Evers, Biodiversity Research Institute; C. Perkins, Univ of Connecticut, Center for Environmental Sciences and Engineering; R. Taylor, Texas A&M Univ, Trace Element Research Laboratory; C. Ramos, College of William & Mary. Many bodies of water around the world are contaminated with mercury from historic industrial and mining activities or ongoing atmospheric deposition, resulting in numerous fish consumption advisories. In contrast, concerns about mercury have only rarely led to waterfowl consumption advisories. In contrast with fish, waterfowl are free to disperse, so hunters and wildlife managers do not know whether waterfowl at a pristine site have spent time at a contaminated site elsewhere. We sampled tissue mercury concentrations of mallards, wood ducks, and Canada geese at a site contaminated with mercury, during the breeding and hunting seasons. We found that most mallards, some wood ducks, and very few Canada geese, had bioaccumulated mercury to levels that posed a risk to their health and the health of hunters who might consume them. We also documented through leg band recovery records that mercury-exposed waterfowl from this contaminated site were harvested by hunters, both locally and as far as 1054 km away. Our results suggest the need for more proactive sampling of waterfowl for mercury, and likely other bioaccumulating contaminants, in order to allow hunters to make more informed choices about consumption risks.

**513 Immunological and Reproductive Health Assessment in Herring Gulls and Black-crowned Night Herons in the Hudson Raritan Estuary** K. Grasman, Calvin College, Dept of Biology, Calvin College, Dept of Biological ; K. Echols, T. May, P. Peterman, R. Gale, C. Orazio, US Geological Survey, Columbia Environmental Research Center. Previous studies have shown inexplicable declines in breeding colonial waterbirds within western New York, New Jersey Harbor between 1996-2002, and elevated concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated biphenyls (PCBs) in double-crested cormorant (*Phalacrocorax auritus*) eggs. This study assessed the immunological health and reproductive success of herring gulls (*Larus argentatus*) and black-crowned night herons (*Nycticorax nycticorax*) in lower New York Harbor during 2003 and evaluated associations between these endpoints and contaminants. In pipping gull embryos, lymphoid cells were counted in the thymus and bursa of Fabricius (sites of T and B lymphocyte maturation). The phytohemagglutinin (PHA) skin response assessed T cell function in gull and heron chicks. In vitro lymphocyte proliferation was measured for adult and pre fledgling gulls. Reference data came from the Great Lakes and Bay of Fundy. Reproductive success of gulls was poor, with only 0.68 and 0.5 chicks/nest surviving to three and four weeks after hatch, respectively. The mean number of developing lymphoid cells was reduced 51% in the thymus and 42% in the bursa of gull embryos from New York Harbor. In vitro lymphocyte assays demonstrated reduced spontaneous proliferation, reduced T cell mitogen-induced proliferation, and increased B cell mitogen-induced proliferation in gull chicks from New York Harbor, but no alterations in adults. The PHA skin response was suppressed 70-80% in gull and heron chicks. Strong negative correlations ( $r = -0.95$  to  $-0.98$ ) between the PHA response and dioxins and PCBs in gull livers was strong evidence to suggest that these chemicals contribute significantly to immunosuppression in New York Harbor waterbirds.

**514 Dietary Exposure of Mink (*Mustela vison*) to Fish from the Upper Hudson River, New York, USA: Effects on Reproduction, Offspring Growth and Mortality** S.J. Bursian, Michigan State Univ, Dept of Animal Science; J.W. Kern, R.E. Remington, Kern Statistical Services, Inc.; J.E. Link, Michigan State Univ, Dept of Animal Science; S.D. Fitzgerald, Michigan State Univ, Dept of Pathobiology and Diagnostic Investigation. The upper Hudson River is contaminated with polychlorinated biphenyls (PCBs) from Ft. Edward, NY, USA to New York City. The major sources of PCBs were capacitor manufacturing facilities at Ft. Edward and Hudson Falls, NY. Field studies indicate that wild mink (*Mustela vison*) collected in the vicinity of the Hudson River have hepatic concentrations of PCBs equivalent to concentrations associated with reproductive impairment in ranch mink experimentally exposed to PCBs. The mink is a commonly selected ecological receptor in studies at sites involving aquatic habitats with elevated concentrations of PCBs. A study was conducted to evaluate the health effects of feeding ranch mink diets containing PCB-contaminated fish from the Hudson River. In the present report, the effects of PCBs on adult reproductive performance and offspring growth and mortality through



31 wks of age are discussed. Diets contained 2.5% to 20% Hudson River fish, providing 0.72 to 6.1  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (5.1 to 38 pg toxic equivalents [TEQs]/g feed). The number of stillborn kits per litter was significantly increased by dietary concentrations of 4.5  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (28 pg TEQs/g feed) and greater. Mortality of 6-wk-old kits was significantly increased by dietary concentrations of 2.8  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (19 pg TEQs/g feed) and greater and body mass was significantly reduced by 1.5  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (9.9 pg TEQs/g feed) and greater. Between 6 and 10 wks of age, 4 (7.8%), 6 (20%), 33 (87%), 8 (89%), 15 (100%), and 2 (100%) animals in the control and 0.72, 1.5, 2.8, 4.5 and 6.1  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed groups, respectively, died. The 7 juvenile deaths that occurred from 10 wks of age to termination of the trial (31 wks of age) were 1 in the 0.72  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed group, the remaining 5 in the 1.5  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed group and the remaining juvenile in the 2.8  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed group. The dietary concentration predicted to result in a 20% increase in kit mortality (LC20) at 6 wks of age was 0.34  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (2.9 pg TEQs/g feed). The corresponding maternal hepatic concentration was 0.80  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  liver (13 pg TEQs/g liver). The conclusions and opinions presented here are those of the authors and do not represent the official position of any of the funding agencies, the Hudson River Trustees or the United States.

**515 Endocrine Disruption in Smallmouth (*Micropterus dolomieu*) and Largemouth Bass (*M. salmoides*) on National Wildlife Refuges in the Northeast USA** A.E. Pinkney, US Fish & Wildlife Service, Chesapeake Bay Field Office; C.P. Guy, USFWS, Chesapeake Bay Field Office; L.R. Iwanowicz, V.S. Blazer, USGS, Leetown Science Center; A. Major, K. Munney, USFWS, New England Field Office; A. Secord, USFWS, New York Field Office; S. Mierzykowski, USFWS, Maine Field Office; S. Lingenfelter, USFWS, Virginia Field Office; C. Stern, T. Kubiak, USFWS, New Jersey Field Office; K. Patnode, USFWS, Pennsylvania Field Office. Members of our team previously reported a high (33% to 100%) prevalence of intersex, specifically testicular oocytes, in smallmouth bass (*Micropterus dolomieu*) in the Potomac River basin, USA. From 2008 through 2010, the US Fish and Wildlife Service and US Geological Survey conducted an On-Refuge Investigation on National Wildlife Refuges (NWRs) in the Northeast USA. Our goal was to evaluate the extent and magnitude of endocrine disruption (including intersex) in smallmouth and largemouth bass (*M. salmoides*) in rivers and impoundments on or adjacent to 21 NWRs. If possible, river sampling included an upstream/downstream design. One area was near a suspected source of endocrine-disrupting chemicals such as wastewater treatment plants (WWTPs) and pulp and paper mills and the other area was remote. Ten male and ten females were captured and necropsied per area. Endpoints were gonad histopathology (including intersex), and plasma hormone and vitellogenin (Vtg) concentrations. Grab samples of ambient water or WWTP effluent were collected, extracted and screened for total estrogenicity via a bioluminescent yeast estrogen screen. Intersex was observed in male smallmouth bass at all sites; however the prevalence and severity differed, in some cases substantially between upstream and downstream sites. Intersex was more prevalent in smallmouth vs. largemouth bass. Vtg was infrequently detected in the plasma of male fish; however, at some locations the median concentration exceeded 1 mg/ml indicating exposure to estrogens. Neither intersex prevalence nor severity correlated with plasma Vtg. Estrogenicity was only above the level of detection in WWTP effluent samples. We will utilize the results to advise refuge managers on land management issues and in commenting on permits for new and expanded point sources.

**516 The Montara Well Release – One Year of Fish Health Monitoring** C. Rawson, Curtin Univ, Dept of Environment and Agriculture; M. Gagnon, Curtin Univ, Dept of Environment and Agriculture, Curtin Univ (Bentley Campus), Dept of Environment and Agriculture, Curtin Univ, Environment and Agriculture. From August to October 2009 an estimated 22,800 barrels of oil and gas condensate were accidentally discharged from the Montara well head to the Timor Sea raising immediate economic, political and environmental concerns. The spill was controlled after seventy-four days, after which a monitoring program to assess the extent of environmental impacts of the well release was enacted. As part of this program, commercially important fish species were collected during three sampling trips spanning 12 months following the release. A suite of physiological indices (condition factor, liver somatic index and the gonadosomatic index), biomarkers (biliary polycyclic aromatic hydrocarbon (PAH) metabolites, liver integrity measured by serum sorbitol dehydrogenase activity (SDH), oxidative DNA damage) and histological examination were used to evaluate

the short and long-term impacts of exposure to petroleum hydrocarbons on fish health. In presenting these results we discuss the continuing importance of ecotoxicology in establishing rapid methods for testing the short- and long-term impacts of acute exposure to industrial contamination events related to the expanding Australian resource sector.

**517 A Transferable Measurement Matrix for Assessing Injury Using an Ecosystem Approach** D. Evers, Biodiversity Research Institute; D. Cristol, College of William and Mary; A. Condon, J. Schmerfeld, US Fish and Wildlife Service; D. Yates, A. Jackson, L. Savoy, Biodiversity Research Institute. The Natural Resource Damage Assessment and Restoration (NRDAR) on the South River, Virginia is a collaborative effort between the Trustees and the responsible party that has followed an ecosystem approach in evaluating the potential injury to trust resources from the historical release of mercury into the river system. Various taxa were examined, emphasizing species within invertivore and piscivore foodwebs. Multiple researchers and experts have been involved in these investigations, and through the NRDAR process, have also been able to answer basic research questions related to mercury exposure and effects in wildlife. Researchers have studied reproductive endpoints, growth and development, behavior, and other sublethal effects. While the primary intent of an NRDAR investigation is to quantify injury in a particular case, the approaches and findings have potential to be more widely applied. As a result of these studies, we have developed a transferable, measurement matrix that is adaptable for assessing injury based on differing taxa, foraging guilds, and other spatially-explicit attributes of potential NRDAR sites across the United States.

**518 Predicting the Sensitivity of Avian Species to the Embryotoxic Effects of Polychlorinated Biphenyls (PCBs) Using a Luciferase Reporter Gene Assay** G.E. Manning, R. Farmahin, Univ of Ottawa, Dept of Biology; S.P. Jones, D. Crump, Environment Canada, National Wildlife Research Centre; S.W. Kennedy, Univ of Ottawa, Dept of Biology, University of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre. Avian species differ in sensitivity to the embryotoxic effects of polychlorinated biphenyls (PCBs). This complicates environmental risk assessments for these chemicals. Avian toxic equivalency factors (TEFs) have been established by the World Health Organization and are applied in the risk assessment of mixtures of dioxin-like compounds (DLCs), including polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and certain PCBs. However, TEFs do not account for differences in species sensitivity to DLCs, and may not accurately predict DLC toxicity in all avian species. A luciferase reporter gene (LRG) assay was developed in our laboratory to measure aryl hydrocarbon receptor (AHR)-mediated cytochrome P4501A (CYP1A) induction in COS-7 cells transfected with different avian AHRs. The assay reliably predicted the embryotoxicity of PCDD/F congeners in 3 model avian species with varying sensitivity to DLCs. Although other assays have been used to measure CYP1A induction using primary hepatocyte cultures, the LRG assay can predict DLC toxicity in rare and endangered species since it requires only the AHR sequence of the species of interest. In the present study, the LRG assay was used to predict 1) the sensitivity of 76 avian species to the embryotoxic effects of PCB 126 and 2) the potency of PCB 126 relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in these species of interest. COS-7 cells were transfected with AHRs representative of different avian species and dosed with 0.003 to 3000 nM PCB 126 or TCDD. Concentration-response curves for luciferase activity in COS-7 cells transfected with 9 of the 15 AHRs of interest have been characterized and the results indicate that avian species sensitivity to CYP1A induction by PCB 126 is dependent on the identity of 2 amino acids present in the AHR ligand binding domain. These results confirm previous findings that avian species can be grouped into 3 general classes of sensitivity to DLCs based on the identity of these amino acids. Future studies will investigate differences in species sensitivity to the effects of PCB 77, 105 and 118. The data from this study regarding the sensitivity of wild avian species to PCB-induced transactivation will be useful in site-specific risk assessments of DLC-contaminated sites.

**519 Differential Expression of Liver Regulatory Genes with Embryonic PCB Exposure** M.E. Barton, Univ of Maryland, Animal and Avian Sciences; K. Dean, Univ of Lethbridge, Neuroscience, Univ of Maryland, post-doctoral research fellow; M. Ottinger, Univ of Maryland, Dept of Animal and Avian Sciences. Biomarkers of exposure and effect provide valuable indices of potential damage to an organism or population exposed to



environmental contaminants. However, wild populations are often exposed to complex mixtures of compounds and there are life stage and species differences in sensitivity. Several well-established biomarkers, such as the induction of hepatic cytochrome P450 monooxygenases, have been used to measure exposure. Few data are available on the use of gene expression for selected responsive genes as a sensitive and biologically meaningful index of PCB exposure. Previously, we reported results using a customized microarray in which hepatic gene expression profiles were examined in response to embryonic PCB exposure in Japanese quail. These data were indicative of potentially useful biomarkers of both exposure and effect. Differentially expressed genes were those involved in glycolysis, xenobiotic metabolism, replication, protein degradation, and tumor regulation, including cytochrome P450 1A5, cytochrome b5, NADH-cytochrome b5 reductase, glutathione-S-transferase, fructose biphosphate aldolase, glycogen phosphorylase, carbonic anhydrase, and DNA topoisomerase II. Because these physiological functions are potential targets of PCB exposure, this current study compared effects of embryonic exposure to PCB 126, PCB 77, or two environmentally relevant mixtures of PCB congeners on gene expression in Japanese quail liver. Embryos were dosed in ovo on embryonic day 0 via air cell injections, with comparative untreated, sham, and vehicle control groups. Embryonic death was tracked during incubation and eggs were allowed to hatch. Hatchlings were sacrificed within 24 hours of hatching; livers were immediately collected, snap frozen on liquid nitrogen, and stored at -80°C until RNA extraction. Expression levels for genes of interest were screened using real time quantitative polymerase chain reaction (qPCR). An assay for ethoxyresorufin-O-deethylase activity was also run for confirmation of induction of this canonical downstream biomarker. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees or the United States. Support from US Fish and Wildlife Service and the Hudson River Trustees.

**520 Expression Pattern of DNA Damage Response Genes Revealed by 55K Microarray Upon UV-B Irradiation in the Copepod, *Tigriopus japonicus*** J. Lee, Hanyang Univ Graduate School, Dept of Chemistry.

Ultraviolet-B (UV-B) radiation affects the genome stability of aquatic organisms by absorption of certain wavelength at the molecular level. Recently, extensive gene information has been identified from the intertidal copepod, *Tigriopus japonicus*. Here, we developed a 55K (54,254 genes) oligomicroarray and tested its usefulness to identify the effect of single dose of UV-B irradiation (12 kJ/m<sup>2</sup>) on transcriptomes of the copepod *T. japonicus*. A total of 35,361 spots were identified to be significantly modulated on the 55K oligomicroarray by hierarchical clustering after exposure to UV-B irradiation over 48 h (6, 12, 24, and 48 h). Of them, 1,300 and 588 genes were observed to be up-regulated and down-regulated at all time points, respectively. Particularly, it was observed that several genes involved in DNA repair mechanism were significantly modulated in the UV-B-exposed *T. japonicus* by microarray and quantitative real-time RT-PCR analysis. In detail, UV-B irradiation specifically up-regulated some genes in non-homologous end-joining (NHEJ), homologous recombination (HR), base excision repair (BER), and mismatch repair (MMR) pathways. On the other hand, a majority of down-regulated genes were representatives for the nucleotide excision repair (NER) mechanism. These results demonstrated that DNA damage would be induced by UV-B irradiation in this species, resulting in reliable induction or repression of various DNA repair mechanism on UV-B-induced DNA damage. In this report, we suggest that a high density microarray-based approach for risk assessment of UV-B irradiation would be useful to elucidate the mechanistic analysis in a non-model organism. This study could also provide a better understanding of molecular mechanisms of cellular protection against UV-B-induced stress.

**521 Al<sub>2</sub>O<sub>3</sub> Nanoparticles Effect the Growth, Development, and miRNA Expression Profile in *Nicotiana tabacum*** C. Burklew, East Carolina Univ, Dept of Biology; B. Zhang, East Carolina Univ, Dept of Biology, East Carolina Univ, Biology, East Carolina Univ, Dept of Biology. Nanoparticles

are small objects that are approximately 1 to 100 nanometers in diameter and are being increasingly used in industry due to their unique properties such as their small size and large surface area. Nanoparticles can be used in coatings for products, colorants, food additives, pharmaceuticals, cosmetics, and paints, therefore residues from their use may be increasingly released into the environment as the use of these products also increases. Currently, nanoparticles have become one major environmental issue and have become

a major environmental pollutant. However, to date, few experiments have been conducted to investigate the effect nanoparticles may have on plant growth and development. It is important to study the effects nanoparticles have on plants and its molecular mechanism because they are stationary organisms that cannot move away from environmental stresses like animals can, therefore they must overcome these stresses by molecular routes such as altering gene expression. microRNAs (miRNA) are a newly discovered, endogenous class of post-transcriptional gene regulators that function to alter gene expression by either targeting mRNA for degradation before it is translated or by preventing the ribosome from translating mRNAs into proteins. miRNAs have been shown to mediate abiotic stress responses such as drought and salinity in plants by altering gene expression, therefore our aim in this study was to classify if certain miRNAs play a role in plant response to Al<sub>2</sub>O<sub>3</sub> nanoparticle stress. In this study we exposed *Nicotiana tabacum* (tobacco) plants (an important cash crop as well as a model organism) to 0%, 0.1%, 0.5%, and 1% Al<sub>2</sub>O<sub>3</sub> nanoparticles and found that as exposure to the nanoparticles increased, the average root length, leaf count, and the germination rate of the seedlings significantly decreased. We also found that miR395, miR397, miR398, and miR399 showed an extreme increase in expression during exposure to 1% Al<sub>2</sub>O<sub>3</sub> nanoparticles compared to the other treatments and the control, therefore these miRNAs may play a key role in mediating plant responses to heavy metal stress in the environment. The results of this study show that Al<sub>2</sub>O<sub>3</sub> nanoparticles have a negative effect on the growth and development of tobacco seedlings and that miRNAs may play a role in the ability of plants to withstand heavy metal stress in the environment.

**522 Identification of Whole Cytochrome P450 (CYP) Genes from the Copepod, *Tigriopus japonicus* and Their Modulation Upon Exposure to Pollutants** J. Lee, Hanyang Univ, Dept of Chemistry, Hanyang Univ Graduate School, Dept of Chemistry; D. Hwang, Hanyang Univ, Dept of Molecular and Environmental Bioscience. The cytochrome P450 superfamily

is a large and diverse group of enzymes. P450 gene expression, protein levels, and P450 mediated metabolism of xenobiotics are induced by PAHs in marine invertebrate species. Thus, the regulation of P450 enzyme activity may play a central role in the adaptation of animals to environmental pollutants. To understand the effects of marine pollutants and the cellular responses, we identified a nearly entire set of the cytochrome P450 (CYP) gene superfamily in the intertidal copepod, *Tigriopus japonicus*. To obtain *T. japonicus* CYP genes, we searched for CYP genes from *T. japonicus* local genome databases (25.56 Gb) sequenced by the genome analyzer, GS-FLX-Titanium and Solexa. We obtained 34 full length CYP genes from *T. japonicus*. To determine the phylogenetic placement of CYP genes, we performed a phylogenetic analysis using maximum likelihood and the Bayesian method comparing with diverse other organisms. The *T. japonicus* CYP genes were clustering with well known vertebrate CYP clans, CYP2, 3, 4, and mitochondrial clans, respectively. We further analyzed specific expression patterns of 34 CYP transcripts in developmental and different sexual stages. Expression patterns of CYP transcripts after exposure to marine pollutants (e.g., heavy oil spillage, endocrine disruptors, and nanoparticles) were also analyzed by quantitative real-time RT-PCR. Differentially modulated expression patterns of CYP transcripts suggest that these invertebrate CYPs are involved in defenses and detoxification mechanisms against marine pollutants. Overall, this is the first report to characterize nearly the entire CYP gene superfamily expressed in this marine copepod. Our studies are helpful in expanding knowledge on the innate roles of CYPs in the metabolism of xenobiotics and in the physiology of marine invertebrate in general.

**523 Differential Life-stage Response to Common Endocrine Disruptors in Two Endangered Species, Atlantic Salmon and Shortnose Sturgeon** T. Duffy, S. McCormick, Conte Anadromous Fish Research Center, USGS. Atlantic salmon (*Salmo salar*) and shortnose sturgeon (*Acipenser brevirostrum*) are endangered anadromous fish that spawn in rivers, and

offspring develop in their natal stream before migrating out to sea or moving into estuaries. Therefore, these species may be exposed to wastewater effluent during early life-history stages, potentially impacting survival and development. Little is known about differential life-stage sensitivity to environmental endocrine disrupting compounds (EDCs) in these species, but this knowledge is crucial to conservation and protection under the Endangered Species Act. The purpose of this research was to determine differential life-stage sensitivity to three common endocrine disrupting chemicals in Atlantic salmon and shortnose sturgeon. We carried out short-term (four-day)

exposures using three doses each of nonylphenol (NP), 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethinylestradiol (EE2) on four life-stages; embryos, yolk-sac larvae, feeding fry and 1+ year-old juveniles of both species. Differential sensitivity was compared using a common biomarker of exposure to EDCs, vitellogenin (Vtg, a precursor egg protein). For each species we validated an enzyme-immunoassay (EIA) measurement of plasma or whole body Vtg and quantitative real-time PCR measurement of Vtg gene transcription. No dose-dependent mortality occurred in our exposures, indicating that sub-lethal impacts are necessary endpoints to determine which life-stages are most sensitive to common EDCs. The impact of exposure to estrogenic compounds on plasma Vtg and Vtg transcription at four developmental stages of Atlantic salmon and shortnose sturgeon will be presented.

**524 Genetic Biomarkers of Developmental Atrazine Exposure Indicate Disruption of Neuroendocrine Function, Cell Cycle Regulation and Carcinogenesis** G. Weber, Purdue Univ, School of Health Sciences; S. Lewis, S. Peterson, M. Sepulveda, J. Freeman, Purdue Univ. Endocrine disrupting chemicals (EDCs) are exogenous agents that alter endocrine system function and are associated with a myriad of diseases. Atrazine, an herbicide commonly applied to agricultural areas throughout the Midwest and a common contaminant of potable water supplies, has been implicated as an endocrine disruptor and potential carcinogen. The specific adverse health effects associated with atrazine exposure and the underlying molecular mechanisms of these effects are not well defined. Several described endocrine disruptors often act as mimetics of ligands or hormones that competitively bind to targeted receptors such as the estrogen and androgen receptors, and subsequently enhance or suppress the corresponding pathway(s). However, to date the molecular mechanism through which atrazine exerts its effects is not fully understood. In an effort to elucidate the toxic mechanisms of action, we exposed zebrafish embryos to 0.3, 3, and 30 parts per billion (ppb) atrazine shortly after fertilization through 72 hours post fertilization (hpf). A portion of the larvae were collected at the end of the treatment period and prepared for global gene expression analysis and morphological analysis. Gene expression data revealed 61 gene targets were significantly altered in the 0.3 ppb concentration while the 3 ppb and 30 ppb concentrations had 202 and 198 gene targets altered, respectively. Interestingly, there was 66% overlap between the 3 and 30 ppb treatment groups with 132 of these gene targets found in both of these treatments. Subsequent gene ontology and molecular network analysis indicated an enrichment of genes associated with neurological disease and neuroendocrine development in the 0.3 ppb gene set. Moreover, in the two higher atrazine treatments (3 ppb and 30 ppb) the altered gene expression profiles were enriched with several gene targets associated with cell proliferation and cancer development. Upon thorough analysis of this genomic data, a subset of genes with prominent roles in reproductive function and cancer has been chosen for targeted analysis in a subset of adult individuals developmentally exposed to atrazine and then allowed to mature under normal conditions. Alteration of these targets in adult individuals will support our hypothesis that developmental exposure to atrazine will elicit a unique transcriptomic signature from which relevant biomarkers of exposure can be identified and used in subsequent molecular analyses.

**525 Is There a Relationship Between Environmental Contaminant Levels, DNA Damage and Fertility Hormones in Bottlenose Dolphins?** E. Pulster, Mote Marine Laboratory, Senior Chemist; L. Schwierzke-Wade, Mote Marine Laboratory; D. Wetzel, Mote Marine Laboratory, Aquatic Toxicology; J. Reynolds, Mote Marine Laboratory. Environmental contaminants are monitored globally, however, the effects of these measured levels are still relatively unknown. Although laboratory tests and selected analyses with wild animals have suggested that exposure toward organic contamination can have significant sublethal effects on marine mammals there has been a lack of empirical data about the effects of organic contaminants on cetaceans. This study attempts to link DNA damage in blood samples as well as levels of fertility hormones in free-ranging male bottlenose dolphins from along the coast of southeastern Georgia (USA) to body burdens of environmental contaminants. Supporting evidence indicates differences in fertility peptide hormone averages for male adults between the two locations along the coast of Georgia, where anti-Müllerian hormone (AMH) concentrations were statistically lower in St. Simons Sound (mean 650 ng mL<sup>-1</sup>) than Sapelo (mean 850 ng mL<sup>-1</sup>). These results suggest potential reproductive impairment in marine mammal populations in close proximity to heavily contaminated areas. Future health assessments of species status or

stock status must include additional investigations into sublethal stressors to properly manage or conserve species.

**526 An Overview of the Current Regulatory Framework for Environmental Assessment of PCPs in the US and the EU** M. Sharma, T. Verslycke, Gradient. In the last decade, several studies have reported concentrations of personal care product (PCP) ingredients in various environmental media, particularly in surface waters. In addition, effects data are increasingly reported in the scientific literature, as greater resources are expended to better understand the environmental safety of "chemicals of emerging concern." In this milieu of increased scrutiny regarding environmental safety, it is appropriate to examine the adequacy of the current regulatory framework for assessing the environmental safety of personal care product ingredients. This presentation will provide an overview of the current regulatory framework for conducting environmental safety assessments of PCP ingredients in the US and in the EU and other existing US/EU regulatory statutes that are relevant in the context of safety evaluations of PCP drug and cosmetic ingredients. We will also evaluate the scientific adequacy/challenges associated with the current regulatory framework and potential regulatory changes in the near future.

**527 The Environmental Risk Assessment of Major Surfactants Used in Consumer Products – History and Watershed Studies Validation** S. Dyer, The Procter & Gamble Company, Central Product Safety, The Procter & Gamble Company, Miami Valley Innovation Center. The surfactants linear alkylbenzene sulfonates (LAS), alkyl sulfates (AS), alkyl ethoxylate sulfates (AES) and alcohol ethoxylates (AE) are among the highest volume and most environmentally studied chemicals used in consumer products. Over the past 3 decades, studies conducted by suppliers, formulators and trade associations (e.g., American Cleaning Institute (ACI), formerly known as the Soap and Detergent Association) have obtained definitive fate and effects data in a variety of environmental media at appropriate levels of biological scale. This presentation consolidates data from lab and field based studies to provide an overall perspective on the environmental risk of each of these chemicals as well as a complex mixture. Such a perspective is especially pertinent in today's scientific climate as these chemicals are often listed as "contaminants of emerging concern". Due the long history of study, it is quite apparent that these chemicals are neither "emerging", that is – new to the world of analytical detection, nor a "concern", that is – pose a significant adverse risk to receiving water biology. These chemicals are designed to clean, hence are sorptive to organic carbon as well as biological surfaces. Due to these properties extensive fate studies have been conducted where their removal in wastewater could be predicted based on laboratory studies and then verified via field-based monitoring studies. Monitoring results provided needed validation data for the development of the national watershed model: GIS-ROUT, recently rebranded by ACI as iSTREEM. Each of these surfactants are typically used in consumer products as mixtures of homologues of various alkyl chainlengths, phenyl positions (i.e., LAS) and ethoxylates (AES and AE). Quantitative structure-activity relationships have been developed that has enabled the assessment of these complex chemicals as mixtures. Further, these QSARs have been verified at the population and community levels via several experimental stream studies. Due to the large effects database, species sensitivity distributions (SSDs) have been also verified via experimental stream studies. The final validation of the safety of these chemicals has come most recently via watershed-based studies where a focus on both overlying water and sediment biota have been studied in the upper Midwest (Indiana, Ohio) and in Texas (Trinity River).

**528 The Environmental Assessment of Fragrance Materials** D. Salvito, Research Institute for Fragrance Materials, RIFM; A. Lapczynski, Research Institute for Fragrance Materials, Inc., Research Institute for Fragrance Materials, Inc, Environmental Specialist. Fragrance materials are used in a wide variety of consumer products. These include soaps and detergents, fabric conditioners, shampoos, cosmetics, fine fragrance, and other personal care products. Among other functions desired by consumers within these products, they often play an important role in signaling to consumers the effectiveness of a product; and, thus, have an impact on the sustainable use of the consumer product as a whole. The environmental risk and hazard of these materials have been studied and reported in the literature (see for example, Salvito, Senna and Federle, 2002). The fragrance materials industry has recently expanded its self-regulatory IFRA Standards to include management measures for environmental risk and hazard. Reported here will be a

summation of testing and research programs to assess environmental impacts of fragrance materials, the application of the IFRA Environmental Standards, and a strategy for progressing this program.

### 529 An Aquatic and Terrestrial Ecological Risk Assessment for Triclosan

T. Barber, ENVIRON International Corp.; M. Bock, P. Fuchsman, J. Lyndall, Environ International; M. Capdevielle, Colgate-Palmolive. Triclosan is an antimicrobial compound used in personal care products in concentrations ranging from 0.1% to 0.3% active ingredient by weight. The primary migration pathway to the environment is via "down the drain" disposal. The conceptual site model included product use, sewage conveyance to wastewater treatment plants, discharge of treated effluent to receiving waters, and production of secondary sewage sludge, which may be applied to land as biosolids. An ecological risk assessment was conducted to evaluate the potential effects of triclosan in the aquatic and terrestrial environments under various exposure scenarios. Given the range of environmental variability, a probabilistic fugacity and bioaccumulation model that explicitly considered the range of potential environmental conditions (e.g., population density, watershed size, wastewater treatment plant removal efficiency, receiving water characteristics, agricultural practices) provided a more complete picture of triclosan in the environment. Predicted environmental concentrations in water, sediment, soil, and tissue were compared to available environmental data. The risk characterization compared the exposure concentrations, body burdens, dietary concentrations, and doses to ecotoxicity benchmarks developed from values published in the open literature and proprietary studies. The comprehensive nature of this work was able to place potential concerns about triclosan in the environment into proper context, identify important exposure factors and data gaps, and prioritize future research needs.

### 530 Simulated Use and Wash-off Release of Decamethylcyclopentasiloxane Used in Personal-care Products

T. Gouin, Unilever, Safety and Environmental Assurance Centre; R. van Egmond, C. Hastie, C. Sparham, Unilever. Given recent concerns regarding the environmental fate of decamethylcyclopentasiloxane (D5), as highlighted by the proposed designation of D5 as persistent and bioaccumulative, as defined under the Canadian Environmental Protection Act, and its potential inclusion in the Canadian Toxic Substances List, assessing the use and potential release to the environment is important for better understanding its environmental safety. A recent Canadian survey reported that D5 is the dominant cyclic volatile methylsiloxane (cVMS) ingredient used in personal-care products (PCPs), with concentrations observed at 680 mg/g in an antiperspirant product. Models that attempt to simulate the use and release of D5 in relation to consumer habits can help identify products that may represent significant sources. In this study, we have performed a sensitivity analysis aimed at better assessing sources of D5 used in PCPs. Probability distributions are defined for inclusion levels of D5 used in different PCP categories, varying from categories defined as leave-on, such as skin creams, antiperspirants, and deodorants, to wash-off, such as liquid soaps, shampoos and conditioners. This information is then combined with knowledge about consumer habits to quantify distributions of amounts being discharged to waste-water treatment plants. This analysis thus provides an assessment of product categories and consumer habits that contribute the most to influent levels.

### 531 Determination of the Levels of Synthetic Musk Compounds in the United States National Rivers and Streams Using Gas Chromatography/Mass Spectrometry

L.I. Osemwengie, United States Environmental Protection Agency, Environmental Chemistry Branch, United States Environmental Protection Agency, National Exposure Research Laboratory; W. Sovocool, United States Environmental Protection Agency, Environmental Chemistry Branch; J. Lazorchak, United States Environmental Protection Agency, Ecological Exposure Research Division; A. Batt, United States Environmental Protection Agency. In 2008-2009, the USEPA and their partners collected fish during a probabilistic survey of 700 navigable waters in the contiguous US under the National Rivers and Streams Assessment program (NRSA), a subset of which were 183 urban river sites. Urban river site fish fillets were analyzed for the occurrence and extent of selected contaminants of emerging concern (CEC), including pharmaceuticals and personal care products. Fish fillets were analyzed for synthetic musk compounds to estimate the potential human exposure during consumption of fish collected from navigable waters of the United States. Approximately 2 g of fish homogenate was surrogate fortified and accelerated solvent extracted with alumina in a stainless steel cell. Gel permeation chromatography was then

performed on each fish sample extract. The resultant extracts were then subjected to a further cleanup step using amino propyl cartridges (SPE). These samples were extracted and analyzed by GC/MS for select synthetic musk compounds and nitro musk metabolites, i.e., Galaxolide, Tonalide (polycyclic musks), Musk Xylene, Musk Ketone (nitro musk compounds), Amino musk ketone, and 4-amino musk xylene (nitro musk metabolites). Upon analysis of 220 fish fillet samples, the polycyclic musks were found in varied concentrations, 756 ng/g to non-detect, while the nitro musk compounds were found either in low concentrations (

### 532 Expanding the Knowledge on Organic UV Filters in the Environment: Occurrence, Removal and Toxicity

P. Gago-Ferrero, IDAEA-CSIC, Environmental Chemistry; M. Badia-Fabregat, UAB; A. Olivares, B. Pina, IDAEA-CSIC, Jordi Girona, Jordi Girona; P. Blaquez, UAB, Enginyeria. Química, Jordi Girona; T. Vicent, UAB; G. Caminal, IQAC (CSIC-UAB), Biocatàlisi Aplicada, Jordi Girona; S. Diaz-Cruz, IDAEA-CSIC, Environmental Chemistry; D. Barcelo, ICRA, Environmental Chemistry, ICRA, Catalan Institute for Water Research, IDAEA-CSIC, Environmental Chemistry Dept. Modern societies depend on a large number of chemicals. Once discharged from industrial and urban sources, they may ultimately enter wastewater. Most of them may be to some extent eliminated during water treatment. However, those not removed may be present in residual concentrations in the receiving ecosystem. Among them, organic UV absorbing compounds are of particular interest due to the raised concern about harmful effects of sunlight; they have been increasingly used in the last decades. This group of additives is worldwide used in many personal care products (sunscreens, cosmetics, hair sprays), in which they amount up to 25% of the total product weight. These chemicals enter the environment either indirectly, via WWTP effluents, or directly, through human recreational activities. There is still limited information about the occurrence, fate and effects of UV filters in the environment, although several of them are known to display estrogenic activity, affect the thyroid axis and reproduction. Moreover, it is documented that UV filters can be bioaccumulated in organisms and concentrated on solid matrices. In this study, environmental concentrations of largely used UV filters and their metabolites were determined, highest levels corresponding to sewage sludge samples with concentrations up to 9.17 ug/g dw. Since biological conventional treatments in WWTP are not effective at degrading most UV filters, new approaches need to be tested. Two of them are assessed in the present work for their elimination. Photodecomposition might be regarded as one of the most important abiotic factors affecting the fate of sunscreen agents in the environment and photocatalysis has been suggested as an effective method to degrade organic pollutants. Biodegradation by the fungus *Trametes versicolor* is considered as a promising alternative to conventional aerobic bacterial degradation, as it is able to metabolize a wide range of xenobiotic compounds. Results obtained by applying both treatments evidenced the high degradation yields achieved by the biodegradation approach. Toxicological assays performed to determine the endocrine disrupting and dioxin-like activities of selected UV filters and their metabolites, indicated that lower EC50 values were obtained for degradation products, ranging from 0.058 to 0.61 mg/L. The toxicity profile upon the biological treatment was also estimated. The outcome demonstrated the suitability of the method to eliminate the estrogenic activity.

### 533 Effects of a Chronic Lower Range of Triclosan Exposure to Stream Mesocosm Communities

C. Nietch, USEPA, Office of Research and Development, National Risk Management Laboratory, Water Supply Water Resources Division, US Environmental Protection Agency, Water Supply Water Resources Division; E. Quinlan, Georgia Gwinnett College; J. Lazorchak, United States Environmental Protection Agency, Ecological Exposure Research Division; C. Impellitteri, US Environmental Protection Agency, Water Supply and Water Resources Division. An environmentally relevant range of doses for the common antimicrobial agent triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) were selected for exposing stream mesocosm communities after a 28 day colonization period with water from a natural waadeable stream. Two mesocosms served as controls while five others were exposed to 0.1, 0.5, 1.0, 5.0, and 10 ug/L Triclosan. The dosing period lasted for 56 days, was designed to simulate a continuous, therefore, chronic point-source discharge to a receiving stream potentially already stressed from organic/nutrient enrichment during the summer low-flow period, and had continuous through-flow with potential for biotic renewal. Sixty six biotic-abiotic parameters were measured throughout the study to provide a comprehensive assessment of triclosan's potential impact on stream biotic



structure and system function. Triclosan's antimicrobial properties were observed first in a dose-response relationship for periphytic bacteria cell densities, but this was not significant until 21 days of exposure. The effect persisted for an additional seven days, but by the 35th day a proliferation in cell densities had occurred across all mesocosms. This coincided with a seemingly natural increase in the periphytic cyanobacteria abundance. By the end of the dosing period bacteria cell densities along with cyanobacteria appeared stimulated in the low doses but were significantly depressed in the two high doses relative to the controls. Similar was the trend across doses for periphytic chlorophyll, ash free dry mass, and nematode densities. The changes to periphytic structure were not significant enough to produce concomitant or subsequent effects in nutrient biogeochemical-based functional indicators, community oxygen metabolism, or the inhabiting macroinvertebrates. However, endpoints for a litter-bag decomposition assay suggested lower fungal biomass, decreased import of nitrogen, and overall lower decay rate in doses 1.0 ug/L and above. Antibiotic resistance increased over the controls was found to be different at concentrations as low as 1 ug/L. Taken together the results suggest that persistent low-levels of triclosan exposure have ecologically relevant consequences for the base of the stream food chain but these may not cascade for significant functional relevance in already stressed systems.

**534 Modeling and Measurement of Climatic and Biologic Control of Gaseous Air-forest Canopy Exchange of Semivolatile Organic Contaminants** J.A. Perlinger, Michigan Technological Univ, Dept of Civil & Environmental Eng.; L. Nizzetto, Norwegian Institute for Water Research; M.D. Rowe, US Environmental Protection Agency. Semivolatile organic contaminants enter the environment through primary emissions, but due to their persistence and volatility, once in the environment they are susceptible to multiple volatilization-deposition steps with environmental media. The surfaces presented to the atmosphere by canopies are ideal for passive uptake and re-emission of the chemicals, and these processes are controlled by climatic and biologic factors. Here, the controls are assessed through implementation and validation of a chemical partitioning model in a physiological and energy balance model for forest canopies. Diurnal and seasonal variability in air-canopy exchange flux, net yearly transfer of chemical from air to canopy, and the forest filter effect under potential climate and land use management scenarios are examined. In addition, the flux estimates are used to determine the chemical sensor resolution required to measure air-canopy exchange flux using the modified Bowen ratio and relaxed eddy accumulation micrometeorological techniques.

**535 Air-Water Exchange Fluxes and Mass Transfer Coefficients for PCBs on the Hudson River** A.L. Sandy, Rutgers, the State Univ of New Jersey, Dept of Environmental Science; L. Rodenburg, Rutgers, the State Univ of New Jersey, Dept of Environmental Sciences; J. Guo, R.J. Misiewicz, Rutgers, the State Univ of New Jersey, Dept of Environmental Science; W.R. McGillis, Columbia Univ, Lamont-Doherty Earth Observatory. In this study, micrometeorological approaches, using turbulent transport measurements, were used to calculate air-water exchange fluxes and mass transfer coefficients (MTCs) for polychlorinated biphenyls volatilizing out of the heavily contaminated Hudson River. The study was conducted during July 2008 on the Hudson River estuary near the Tappan Zee Bridge. Volatilization PCB fluxes were calculated using the modified Thornthwaite-Holzman equation. Values of friction velocity and atmospheric stability were calculated using the Aerodynamic Gradient and Eddy Correlation techniques. MTCs were determined using these turbulent fluxes divided by the corresponding dissolved phase concentrations. The median MTCs ranged from 0.29 for hexachlorobiphenyls to 2.2 m/d for monochlorobiphenyls with a propagated uncertainty of about 70%, lower than in previous studies. These field measurements differ by as much as a factor of 23 from predictions based on the widely-used Whitman two-film model.

**536 "Old" and "New" Flame Retardants in the Great Lakes Basin** A. Salamova, Indiana Univ, School of Public and Environmental Affairs, Indiana Univ, SPEA; M. Venier, Indiana Univ, School of Public and Environmental Affairs, Indiana Univ; R.A. Hites, Indiana Univ, School of Public & Environmental Affairs. Amina Salamova\*, Marta Venier, and Ronald A. Hites School of Public and Environmental Affairs, Indiana Univ, Bloomington, Indiana 47405 USA Email contact: asalamov@indiana.edu Due to environmental concerns, the use of most polybrominated diphenyl ethers (PBDEs) was banned by the European Union and phased out in

the United States in 2004. Perhaps as a result, there seems to be a shift to using non-regulated flame retardants, and several "new" flame retardants have been recently detected in the environment. Here we present temporal and spatial trends in SPBDE, decabromodiphenylethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy)ethane (TBE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB) concentrations in air (vapor and particle phases) and in precipitation at the five United States Integrated Atmospheric Deposition Network sites located in the Great Lakes basin. These samples were collected from January 2005 to December 2009, inclusive. Overall, Chicago and Cleveland had the highest concentrations of SPBDEs in both air and precipitation, suggesting a strong urban atmospheric source of these pollutants. This urban effect could also be observed at the rural Sturgeon Point site. The other two remote sites, Sleeping Bear Dunes and Eagle Harbor, have the lowest concentrations of these contaminants. Following this pattern, TBE and DBDPE have highest levels at urban Chicago and Cleveland. The spatial pattern for HBB and PBEB was surprising: highest levels of these compounds were detected at the rural site in Sturgeon Point and at the remote site in Eagle Harbor, respectively. The sources of HBB and PBEB at these sites are not known. The temporal trends of SPBDEs, DBDPE, HBB, PBEB, and TBE were investigated using harmonic regression applied to concentrations in all three phases at all the sites combined together, which allowed us to look at the overall temporal behavior of these compounds in the Great Lakes region.

**537 Polybrominated Diphenyl Ethers (PBDEs) in the Atmosphere Surrounding Two WWTPs in Tuscany (Italy)** A. Cincinelli, Univ of Florence, Chemistry Dept; T. Martellini, Univ of Florence; K. Jones, LEC, Lancaster Univ. Polybrominated diphenyl ethers (PBDEs) constitute an important group of brominated flame retardants that have been extensively produced and used in numerous everyday products. The market demand for PBDEs has been dominated by three major industrial formulations (penta, octa, deca-BDE mixtures), which are mixtures of tetra- to deca-congeners at various proportions. The penta and octa formulations were banned in Europe in 2004, deca-BDE was also prohibited by July 2008. PBDEs enter the waste water treatment plants (WWTPs) from a variety of sources including urban and agricultural run-off, domestic wastewater, industrial point discharges, etc. and can be released to the atmosphere by volatilization and aerosol formation generated by bursting bubbles produced by the diffused aeration systems that provide oxygen to the microbial flora in the activated sludge processes. Thus, wastewater treatment processes are often overlooked as responsible for the emission of PM10 and associated pollutants. In this study, we collected PM10 samples in the atmosphere (downwind and upwind) surrounding two waste water treatment plants (WWTPs) in Tuscany (Italy), between 2004 and 2006, in order to determine the atmospheric occurrence and distribution of PBDEs and investigate WWTPs as source of these pollutants to the atmosphere. PBDEs concentrations were compared to those found in samples taken simultaneously at reference sites that were not influenced by the WWTPs. Particulate and gas-phase samples were also collected to evaluate not only the gas-particle partitioning of PBDE, which is fundamental for assessing their fate and human exposure, but also to evidence temporal variations. Moreover, the risk of potential adverse health risks was calculated using the mean measured concentrations.

**538 Analysis of Veterinary Growth Promoters in Airborne Particulate Matter by Liquid Chromatography Mass Spectrometry** B.R. Blackwell, Texas Tech Univ/TIEHH, Environmental Toxicology, Texas Tech Univ, Environmental Toxicology; Q. Cai, Texas Tech Univ, TIEHH, Environmental Toxicology; M. Buser, Oklahoma State Univ; B. Johnson, Texas Tech Univ, Animal and Food Sciences; G.P. Cobb, P.N. Smith, Texas Tech Univ/TIEHH, Environmental Toxicology. Veterinary growth promoters are widely used throughout the United States in livestock production. In beef production, steroid hormones are administered to an estimated 90% of cattle. These include the synthetic steroids trenbolone acetate and melengestrol acetate, as well 17 $\beta$ -estradiol. Given the endocrine-modulating activity of steroid growth promoters, a sensitive and reliable analytical method is needed to detect trenbolone, melengestrol acetate, estradiol and related residues in environmental matrices. We have developed a method that incorporates solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry for the simultaneous determination of trenbolone, trenbolone, melengestrol acetate, estrone, and estradiol in airborne particulate matter (PM) samples. PM was collected on 47 mm Zefluor filters. Sample preparation involved shaking extraction in methanol followed by cleanup on

Strata-X and Florisil SPE cartridges. Analytes were separated using reversed-phase liquid chromatography on a Kinetex PFP column. Column effluent underwent atmospheric-pressure chemical ionization (APCI) followed by detection using a triple-quadrupole mass spectrometer in SRM mode.  $17\beta$ -trenbolone-d<sub>3</sub>, melengestrol acetate-d<sub>3</sub>, and estradiol-d<sub>5</sub> were used as internal standards. Due to high noise levels interfering with estrone and estradiol determination, estrogens were derivatized using pentafluorobenzyl bromide. Detection limits, recovery, and repeatability of all target compounds will be presented and discussed in detail.

**539 Estimating Hourly Passive Sampler Mass Transfer Rates and Concentrations in an Urban Landscape Using a Numerical Weather Prediction Model** N.T. Petrich, S.N. Spak, Univ of Iowa, Civil & Environmental Engineering; C. Stanier, Univ of Iowa, Chemical and Biochemical Engineering; Z.L. Rodenburg, Univ of Iowa, Civil & Environmental Engineering; G.R. Carmichael, Univ of Iowa, Chemical & Biochemical Engineering; K.C. Hornbuckle, Univ of Iowa, Dept of Civil & Environmental Engineering. Polyurethane foam (PUF) passive air samplers represent an inexpensive and practical way to sample semivolatile organics compounds and their spatial gradients over urban areas. However, canonical methods for estimating concentrations from passive sampling assume constant empirical mass transfer rates and temperature, which add unquantified uncertainties in temporal representativeness to concentrations estimated from raw sampled mass. These methodological choices currently limit the ability to calculate and constrain uncertainty in estimated concentrations, and to directly compare passive sampler concentrations with time-resolved concentrations from active sampling methods and chemical transport models. Here we present an alternative method for estimating hourly flow rates, mass transfer coefficients, and concentrations—including average concentration at a reference temperature—from first principles using hourly meteorology (temperature, wind direction, and wind speed) simulated by the Weather Research and Forecasting (WRF) model. This method is applied to gas and aerosol polychlorinated biphenyl (PCB) congeners at an urban passive sampling network in Chicago, Illinois during 2008. We evaluate the importance of sampler height and orientation, temperature, sub-gridscale turbulent mixing, internal fluid dynamics within the PUF housing, and PUF air concentration gradients. Effects of model spatial resolution are considered using nested WRF simulations on 12 km, 4 km, and 1.33 km grids over the city. Spatial variability in mass transfer coefficients and concentrations are compared with results from canonical empirical methods, and temporal variability evaluated through comparison with contemporaneous concentrations from high volume air samplers.

**540 Modeling Assessment of Environmental Fate, Latitudinal Distribution and Long Range Transport of Decamethylcyclopentasiloxane (D5)** S. Xu, Dow Corning Corporation, Health and Environmental Sciences. Decamethylcyclopentasiloxane (D5), a major ingredient in personal care products, has been shown to be mainly distributed in the air compartment due to its high air/water partition coefficient although small fraction of it may also partition to the freshwater sediment near the point of release. The airborne D5 has an average atmospheric half-life of 6.9 days and thus may travel a long distance in the air. The environmental fate of this chemical in a regional environment has been analyzed by fugacity modeling. Objective of this study was to assess the environmental fate of D5 in a global scale and to determine its global distribution and long range transport potential (L RTP). The environmental fate and global distribution of D5, the travel distance of D5 in air and water, and the deposition of airborne D5 to surface media in the remote region were estimated using both GloboPOP model and a global average chemical fate model: the OECD tool for screening Pov and L RTP (or the Tool). All simulations were conducted using the default values of the model for compartment dimensions and properties. The chemical specific data inputs, including physiochemical properties and the release scenarios, were either experimental values or the best estimation based on the experimental data. Generally, that D5 behaves as a degradable and highly hydrophobic flier with little potential to be transported to, and accumulated in, the remote surface media. For example, > 99 % of D5 was degraded after 10 years of continuous release. The majority (97.7%) of the non-degraded 1% of D5 was distributed in the global atmosphere. The Arctic contamination potential for D5 is > 3.8 orders of magnitude lower than that for Gamma-Hexachlorohexane and PCB 28, two POP reference materials. When all the results from both models were considered, it was concluded that most of D5 will be degraded in three latitudinal zones of the Northern

hemisphere where they are released. Although some of it may be transported to the remote atmosphere such as the Arctic, the airborne D5 has very low potential to be transferred to the remote surface media and ecosystem.

**541 Organosiloxane Compounds in Urban and Rural Air** R. Yu-cuis, Univ of Iowa; K.C. Hornbuckle, Univ of Iowa, Dept of Civil & Environmental Engineering. The organosilicon compounds octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dododecamethylcyclohexasiloxane (D6) are widely used in household goods and personal care products. Due to their prevalence and chemical behavior, cyclic siloxanes have been targeted for study as possible persistent organic pollutants. We have applied active and passive methods to collect airborne siloxanes in urban and rural environments and analyzed the samples using the internal standard method (internal standard tetrakis trimethyl-siloxysilane, M4Q) and gas chromatography with electronic impact mass detection. Quality assurance metrics included sample replicates, field and laboratory blanks, laboratory background measurements, sampling media breakthrough, instrument linearity, and spike recoveries. The method was applied onboard the EPA R/V Lake Guardian, in urban Chicago, and in rural and suburban Iowa. We have found siloxanes to be present in every environment tested but much greater in concentration and variability in urban environments. Concentrations of D4, D5, and D6 over Lake Michigan near Chicago averaged 30, 230, and 9.3, respectively.

**542 How Weirs in Agricultural Drainage Ditches Alter Conditions for Sediment Accumulation and Phosphorus Retention** E. Usborne, Mississippi State Univ, Wildlife, Fisheries and Aquaculture; R. Kroger, Mississippi State Univ, Dept of Wildlife, Fisheries and Aquaculture, Mississippi State Univ. Agricultural phosphorus loads, carried by surface runoff through ditches, contribute to eutrophication. Low grade rip rap weirs potentially reduce phosphorus (P) from entering primary aquatic systems by slowing water flow through drainage ditches, allowing sediments to settle out and P to sorb to soil. Weirs have the potential to alter the pH and hydraulic residence time within the ditch system, thereby affecting P retention by ditch soils. A laboratory experiment was conducted in microcosm chambers to simulate this effect. Ditch sediments collected between Portageville, MO and Vidalia, LA were inundated for three day, three week, and three month treatments. Average pH within the microcosms decreased from 6.960 to 6.876 over three days. Over three weeks and three months, pH increased from 6.960 to 7.075 and from 6.960 to 7.266 respectively. Two field studies were conducted in order to compare experimental data with field data and record sediment deposition rates. One field study, measuring weirs of varying maturity, shows sediment accumulation behind a weir structure to be twice as much as ditches without weirs. A second field study is monitoring individual weirs monthly after installation to detect seasonal differences in weir effects on sediment deposition and P retention. Total P and P fractions are being measured on the sediment samples collected from the lab and field. Preliminary results show longer inundation treatments and older weirs have larger concentrations of iron phosphate. Predicting time periods between soil P saturation and sediment accumulation limits will allow us to inform farm managers of optimal times to dredge ditches for greatest P retention efficiency.

**543 Nature and Estimated Toxicity of Polar Compound Mixtures Measured in Groundwater at Petroleum Release Sites and Reported as TPHd/DRO** D. Zemo, Zemo & Associates; K. O'Reilly, Exponent; R. Mohler, A. Tiwary, R. Magaw, K. Synowiec, Chevron Environmental Technology Company. Both petroleum hydrocarbons and polar non-hydrocarbons are measured as "TPHd/DRO" using EPA Method 8015 unless a silica gel cleanup (SGC) is used. Sources of polar compounds include (1) natural organics, (2) petroleum biodegradation byproducts (metabolites), (3) sampling/lab contamination (e.g., phthalates), or (4) non-petroleum chemicals. Work since the mid 1990s has shown that a large proportion of organics in groundwater at sites with biodegrading petroleum are polar compounds. The polar compounds occur within and downgradient of the source area and within the area of aerobic and anaerobic biodegradation, and therefore have been assumed to be primarily biodegradation byproducts. Some regulatory agencies have been hesitant to allow SGC of samples to separate hydrocarbons from polars because of uncertainty regarding the nature and toxicity of compounds removed by the SGC. This study provides a better understanding of the potential composition of polars in groundwater based on knowledge of metabolic pathways and actual analytical results. This

work summarizes what is known about the human toxicity of the major classes of polar compounds found in groundwater, compiled from numerous published sources. It provides analytical results from groundwater samples collected at multiple sites with biodegrading petroleum that (a) identify and quantify specific polar compounds of potential human toxicity concern; and (b) tentatively identify polar compounds present using two-dimensional gas chromatography mass spectrometry (GCxGCxMS). In summary, this study shows that the polar compounds present are primarily organic acids and alcohols, with lesser amounts of phenols, aldehydes and ketones. The human toxicity of these chemical classes varies from "non-toxic" to "low to moderate". The overall toxicity of the mixture present in groundwater at these sites is characterized as "low" based on the relative proportions of the classes. The mixture of polar compounds present in groundwater at these sites has lower toxicity than dissolved aromatic hydrocarbons, and does not pose a significant risk to human health. Results also confirm that water quality objectives developed based on hydrocarbons should not be applied to the polar compounds, and a SGC should be routinely used to separate hydrocarbons from polar compounds if TPHd/DRO analysis is required.

**544 PAH Reaction Rates with Peroxy-Acid Treatment: Prediction of Reactivity Using Local Ionization Potential** J. Shoulder, C. Brenemann, Rensselaer Polytechnic Institute, Chemistry and Chemical Biology; M. Nyman, Rensselaer Polytechnic Institute, Dept of Civil and Environmental Engineering. Property-Encoded Surface Translator (PEST) descriptors have been found to be highly correlated with the degradation rates of polycyclic aromatic hydrocarbons (PAHs) by the peroxy-acid process. Reaction rate constants ( $k$ ) in  $\text{hr}^{-1}$  for nine PAHs (e.g., acenaphthene, anthracene, benzo[a]pyrene, benzo[k]fluoranthene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene) were determined by a peroxy-acid treatment method that utilized acetic acid, hydrogen peroxide, and a sulfuric acid catalyst to degrade the polyaromatic structures. Molecular properties of the selected nine PAHs were derived from molecular structures optimized at B3LYP/6-31G\* and HF/6-31G\* levels of theory. Properties of molecular energy, adiabatic and vertical ionization potential (IP), highest occupied molecular orbitals (HOMO), HOMO/lowest unoccupied molecular orbital (LUMO) gap energies and HOMO/singly occupied molecular orbital (SOMO) gap energies were not correlated with rates of peroxy-acid reaction. PEST descriptors were calculated from B3LYP/6-31G\* optimized structures and were found to have significant levels of correlation ( $r^2 = 0.787$ ) with  $k$ . PIP Min described the minimum local average ionization potential on the surface of the molecule and was found to be most related to  $k$  of the selected PAHs used in this investigation. PEST technology appears to be an accurate method in predicting reactivity for the selected PAHs and could prove to be a valuable asset in building treatment models and in effective remediation design for PAHs and other organic contaminants in the environment. Therefore, this technique could potentially save time and expense in treatment design and implementation.

**545 Photosensitized Transformation of Acetochlor and Isoproturon by Chromophoric Dissolved Organic Matter** M. Langlois, L. Weavers, The Ohio State Univ, Dept of Civil and Environmental Engineering and Geodetic Science; Y. Chin, The Ohio State Univ, Dept of Geological Sciences. The phototransformation of agricultural products in wetlands is strongly influenced by chromophoric dissolved organic matter (CDOM), a well-studied and important process by which absorbed light causes the production of reactive oxygen species (ROS) in illuminated, aerated waters. We investigated the photolysis of two broadleaf herbicides, acetochlor (AC) and isoproturon (IP), in water from four US wetlands (an agricultural wetland in Ohio [OH], Kawai Nui wetland in Hawaii [HW], the Everglades in FL [EV], and Okefenokee Swamp in GA [OK]). Wetland waters spiked with the target herbicides were irradiated in a solar simulator (Suntest CPS+) and analyte concentrations monitored over time. Further, UV-vis absorbance of CDOM in our samples and dissolved oxygen (DO) were also monitored at time points throughout the experiment to determine the relationship between photobleaching and herbicide degradation. Indirect photolysis of AC was slow; even across chemically diverse waters ([DOC]: 7-190 mg C/L, pH 3.5-9, [Fe] = 1.1-28 mM,  $[\text{NO}_3^-] < 7.5 \text{ mM}$ ) with consistent half-lives at  $t_{1/2} = 1.8 \pm 0.3 \text{ d}$ . Quenching of the reaction by *t*-butanol as well as dramatically higher rates in photo-Fenton-enhanced low-pH water strongly indicate a hydroxyl radical ( $\text{OH}^\bullet$ ) mediated pathway. The similarity of  $\text{O}_2$  consumption between control (no-herbicide) and AC-spiked samples indicate that CDOM is the main source of photochemical oxygen demand in sunlit

waters. Conversely, IP actually contributed both to  $\text{DO}$  loss and to changes in UV-vis absorbance in irradiated waters, indicating that it and DOM are directly involved in  $\text{O}_2$  consumption. These data suggest that  $\text{O}_2$  is involved in quenching activated IP intermediates. Rate constants for both herbicides in these wetland waters do not correlate strongly to any one chemical or optical property of the sampled waters, indicating that it is difficult to predict the photochemical behavior of these type of herbicides based upon the chemical constituents in natural waters.

**546 Environmental Fate of Flame Retardant Chemicals and Triclosan in Biosolids-amended Soil** E.F. Davis, H.M. Stapleton, Duke Univ, Nicholas School of the Environment. Disposal of biosolids as a nutrient-rich soil amendment is a growing trend in the United States. However, these biosolids are not regulated for organic contaminants that may not be fully removed during wastewater treatment; thus, extensive land application of biosolids may enhance the bioaccumulation and transport potential of these compounds. This study was undertaken to determine the fate of chemicals of emerging concern in biosolids-amended soil in a controlled greenhouse experiment. Our objective was to determine whether a suite of brominated and chlorinated flame retardants as well as the antimicrobial agent triclosan would be accumulated by alfalfa (*Medicago sativa*) grown in the amended soil and/or undergo transformation reactions over time. Biosolids were collected from a wastewater treatment plant in Durham, North Carolina in July of 2010. In a 90-day controlled experiment conducted in the Duke Univ research greenhouse in the summer of 2010, pots of soil were amended with biosolids at three different levels (0% (control) and approximately 10% and 30% by volume). Alfalfa was grown in the amended soil and each treatment was prepared in triplicate; unplanted controls were also prepared. At predetermined time points, subsamples of soil and above- and below-ground plant biomass were collected and frozen until analysis. A suite of chemicals have been detected in the biosolids as well as in the three soil treatments from Day 0 of the experiment, including polybrominated diphenyl ethers (PBDEs), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), di(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), Dechlorane Plus and 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan). Triclosan was the dominant contaminant detected in the biosolids ( $3125 \pm 226 \text{ ng/g dw}$ ) followed by PBDEs ( $2784 \pm 180 \text{ ng/g dw}$ ), TDCPP ( $1557 \pm 112 \text{ ng/g dw}$ ), TBPH ( $548 \pm 49 \text{ ng/g dw}$ ), TBB ( $297 \pm 39 \text{ ng/g dw}$ ) and Dechlorane Plus ( $22 \pm 4 \text{ ng/g dw}$ ). Concentrations of triclosan in the amended soil treatments on Day 0 ranged from  $\Sigma$ PBDEs ranged from 2 to  $1687 \text{ ng/g dw}$  and TDCPP ranged from 73 to  $581 \text{ ng/g dw}$ . Soil and alfalfa samples from the remaining time points are currently being analyzed for these compounds and their known degradation products. Contaminant concentrations over time in soil and alfalfa biomass as well as the formation of degradation products will be presented.

**547 Pesticides in Guttation Droplets Following Seed Treatment – Field Studies** D. Schenke, Julius Kühn-Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection; I. Joachimsmeier, J. Pistorius, Julius Kühn-Institute, Institute for Plant Protection in Field Crops and Grassland; U. Heimbach, Julius Kühn-Institute. The European Council Regulation (EC) 1107/2009 defines that plant protection products shall only be approved if under the proposed conditions of use there is no unacceptable risk for honeybees. An exposure assessment is a precondition for the terrestrial ecotoxicological risk assessment. According to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev 2 final) realistic exposure conditions should be taken into account if soil applied systemic plant protection products (e.g., applied as seed dressing) have a very high oral toxicity. Some data are available for transport of systemic compounds in the xylem to pollen or nectar but not for excretion via guttation droplets. Guttation takes place at high humidity and lack of transpiration. The droplets are excreted at the end of the xylem veins at leaf edges. In certain circumstances guttation may be a potential water source for water collecting bees and bees may potentially be exposed to systemic compounds used as seed coating. The frequency and duration of guttation were observed at major crop species under middle European climate conditions at field stations in Berlin and Braunschweig from April to August 2010. Rarely droplets were found on leaves of sugar beet, potato, onion, carrot and pea. The first guttation droplets in maize, cereal, oilseed rape and cucumber were seen at emergence and beginning of leave development. These crops guttated with high frequency. Spring barley and oat showed guttation at leaf edges until booting and winter wheat until the end of heading. Guttation



stopped in winter oilseed rape at fruit development and in spring oilseed rape at emergence of inflorescence. Maize and cucumbers excreted guttation droplets up to the ripening of fruit and seeds. Grass on field boundaries guttated also often combined but good distinguishable with presence of dew. Guttation droplets from agricultural crops sown with pesticide coated seeds were sampled for analysis. Special studies were designed to examine the influence of different rates of pesticides on maize seeds on the concentration of the neonicotinoids imidacloprid, clothianidin and thiamethoxam in guttation droplets. The concentration of clothianidin in guttation water was also analysed after use of the same rates per ha as point (coated seed) and as row in furrow application (granulate).

**548 The Sky is Falling: Evaluating the Potential Impact of Soil Deposition from Static Rocket Testing on Crop Plants** W.J. Doucette, Utah State Univ, Utah Water Research Laboratory; S. Mendenhall, L. McNeill, J. Chard, Utah State Univ. Static testing of reusable solid rocket motors (RSRMs) at the ATK Promontory site can displace large amounts of soil that become entrained in the combustion cloud. As the cloud cools, the soil drops on the surrounding area (typically within a 3-5 mile radius) that includes farmland, rangeland, low-density residential housing and a wildfowl management area. This deposition material, referred to as Test Fire Soil (TFS), is different from the original soils due to its exposure to the elevated temperatures and the aluminum oxide and hydrochloric acid rocket fuel combustion products. Local residents and farmers have observed TFS being deposited on their gardens, fields, and crops and have expressed concerns regarding the potential impact of this material. To address these concerns, we conducted growth and uptake studies in a controlled greenhouse environment. Corn and alfalfa were grown to for a minimum of 45 day or until maturity, in columns of soil with and without the addition of TFS material. Water and fertilizer were applied to simulate local field applications. Leachate if generated was analyzed for Cl, Al and other elements. Three treatments were evaluated: 1) soil without the addition of TFS, 2) soil completely mixed with 5-10% TFS and 3) soil with TFS applied to soil surface after 45 days of plant growth. Analysis of soil and plant tissue was conducted periodically throughout the study to determine if there was a significant difference between crops grown in soil and those exposed to TFS. The potential impact of the TFS on plant health was also evaluated by comparing plant growth and nutrient content between the treatments. In addition to soil uptake, the impact of deposition through direct contact between the TFS and above ground (foliar) portions of the plants was also evaluated.

**549 Volatilization of Trichloroethylene from Trees and Soil: Measurement and Scaling Approaches** W.J. Doucette, Utah State Univ, Utah Water Research Laboratory; H. Klein, J. Chard, B. Bugbee, R. Dupont, Utah State Univ; B. Plaehn, Parsons. Phytoremediation, using fast growing trees like poplars and eucalyptus, has been promoted as a low cost, sustainable remediation alternative for the clean up of shallow groundwater contaminated with volatile chlorinated solvents like trichloroethylene (TCE). Volatilization from leaves and trunk (phytovolatilization) is thought to be a primary mass removal mechanism with trees also reported to enhance soil volatilization. However, quantifying this mass removal on a field scale is challenging. The focus was to develop a simple, low-cost approach for measuring the volatilization of TCE from leaves, trunk and soil and to use the measurements to estimate TCE removal from phytoremediation demonstration sites at Travis and Fairchild Air Force Bases (AFBs). Tree cores, collected using an increment borer and analyzed using headspace chromatography/mass spectrometry (GC/MS), were used to determine the relative TCE concentrations within the plume beneath the trees and estimate the TCE mass within each tree. Volatilization from leaves was measured by enclosing several leaves inside a flow-through glass chamber continuously purged with air to maintain normal transpiration and temperature. Air exiting the chamber was sampled using sorbent tubes and analyzed for TCE by thermal desorption GC/MS. Humidity probes, placed at the chamber entry and exit, were used to determine transpiration. Volatilization of TCE from tree trunk and soil surfaces was measured by enclosing a known area within a small stainless steel chamber. Fans mixed the air in the chamber that was recirculated through sorbent tubes to continuously remove TCE. After a known time interval, the sorbent tubes were analyzed for TCE. Using the volatilization measurements, a Thiessen polygon approach was used to estimate the removal of TCE at Travis (839 g/yr) and at Fairchild AFBs sites (18 g/yr) with the majority associated with leaf and soil volatilization. It was also observed that volatilization of TCE from the soil surface was greater

inside the planted areas than outside. Based on estimates of TCE mass in the groundwater below the sites at Fairchild and Travis AFBs, volatilization removed 5 and 50%, respectively. The lower removal at the Fairchild site was not surprising since the average TCE groundwater concentration was approximately 10 times lower than at the Travis AFB site and the growing season is shorter by a factor of two.

**550 PBDEs, PCBs, and DDT in Resident Backyard Birds of Suburban and Rural Areas Near Washington D.C.** C. Orazio, US Geological Survey, Columbia Environmental Research Center; N. Diggs, Smithsonian Migratory Bird Center, National Zoological Park, Smithsonian Conservation Biology Institute; P. Peterman, US Geological Survey, Columbia Environmental Research Center; S. Tan, C. Grimm, Smithsonian National Zoological Park, Center for Species Survival; K. Feltz, K. Echols, US Geological Survey, Columbia Environmental Research Center; P. Marra, Smithsonian Migratory Bird Center, National Zoological Park, Smithsonian Conservation Biology Institute. Residue levels of polybrominated diphenyl ethers (PBDEs), organochlorine pesticides, and PCBs were measured in eggs of northern cardinals (*Cardinalis cardinalis*) from several neighborhoods surrounding the Washington DC area to determine if there are location-specific exposure patterns for these chemicals in resident birds. The diet of the cardinal consists mostly of fruits and seeds (and water) throughout the year with the summer diet including insects. Eggs were collected in May 2008 from 5-6 first-brood nests within each neighborhood. Neighborhoods included Arlington VA (suburban), Takoma Park MD (suburban), Greenbelt MD (managed suburban community), Dunkirk MD (semi-rural estate homes), West Friendship MD (semi-rural), and Whitehall MD (rural farming community 70 mi NE of DC). Nest locations were generally separated from each other by at least 100 yards from within a 10-20 acre area. Egg contents were solvent extracted, purified, and analyzed by GC/MS and GC-high resolution MS. Within each neighborhood, PBDE concentrations and congener patterns in the eggs were similar. PBDEs 47, 99, 100, and 209 were the predominant PBDE congeners detected in the eggs. Total-PBDE levels in suburban eggs were higher (with the exception of Arlington) as compared to rural areas. The highest levels of PBDE-47 and PBDE-209 were found in an egg from Greenbelt: 3.08 and 3.25 ng/g wet weight (ww) or 57 and 53 ng/g lipid weight basis, respectively. Residues of p,p'-DDE were detected in eggs from all areas, with suburban areas having higher levels that ranged from < 2 to 569 ng/g ww. Levels of p,p'-DDT were below detection (< 0.1) in eggs from the three more rural neighborhoods tested, while p,p'-DDT ranged from 0.5 to 16 ng/g ww with the exception of two suburban eggs: one from Takoma Park with 127 ng/g ww and one from Greenbelt with 1130 ng/g ww. The DDT/DDE ratios in these two eggs (4:1 and 2:1) suggested a recent DDT-contaminated diet, either from foraging in a DDT contaminated area or from DDT-contaminated seed in a backyard bird feeder. We measured egg shell thickness, but saw no correlation to the high DDT levels in these two eggs. Total-PCB in eggs from the three suburban neighborhoods ranged from 11 to 21 ng/g ww, whereas the rural area levels were < 5 ng/g. Levels of chlordane were higher in eggs from older suburban neighborhoods, likely from related to lingering termiticide treatment of home foundations.

**551 Comparison of PCBs in East Chicago, IN and Columbus Junction, IA in Indoor and Outdoor Air** T. Schulz, Univ of Iowa, Dept of Civil & Environmental Engineering, IHR-Hydrosience and Engineering; C. Stanier, Univ of Iowa, Chemical and Biochemical Engineering; P. Thorne, J. Dewall, Univ of Iowa, Occupational and Environmental Health; K.C. Hornbuckle, Univ of Iowa, Dept of Civil & Environmental Engineering. We have deployed and collected polyurethane foam based passive samplers (PAS-PUF) at residential homes in two communities: East Chicago, Indiana and Columbus Junction, Iowa. At each residence, a sampler was deployed inside the home and outside the home. The samples have been extracted and analyzed for the full suite of polychlorinated biphenyls (PCBs). Higher levels were expected to be found in East Chicago compared with Columbus Junction due to a higher level of current and historical industrial activity in Northwest Indiana relative to rural Iowa. However, preliminary results indicate that the two communities do not have statistically significant differences in outdoor air concentrations. Total PCB masses quantified in indoor air are fairly similar to outdoor, but when transformed to air concentrations using published sampling rate constants, they are calculated to be approximately three times the outdoor concentration. Congener distributions are examined

to elucidate sources of PCBs from building materials and/or nearby industrial activity.

**552 Spatial Distribution of Chlordane and PCB Congeners in Soil in Cedar Rapids, Iowa, After a 500-year Flood** A. Martinez, Univ of Iowa, Dept of Civil & Environmental Engineering, and IIHR-Hydroscience and Engineering; N.R. Erdman, Z.L. Rodenburg, Univ of Iowa, Civil & Environmental Engineering; P. Eastling, IIHR- Univ of Iowa Civil and Environmental Engineering, IIHR- Univ of Iowa Civil and Environmental Engineering; K.C. Hornbuckle, Univ of Iowa, Dept of Civil & Environmental Engineering. After the largest flood on record occurred in Cedar Rapids, Iowa (June 2008), we measured chlordane and polychlorinated biphenyls (PCBs) in surficial soils. Chlordane compounds, including trans-chlordane (TC), cis-chlordane (CC) and trans-nonachlor (TN), ranged from 0 to 7500 ng g<sup>-1</sup> d.w., with an average and standard deviation of  $130 \pm 920$  ng g<sup>-1</sup> dry weight (d.w.). These chlordane concentrations are about 100 times larger than background concentrations and could be due to historical residues from residential applications of the chlorinated insecticide. ΣPCBs concentrations ranged from 3 to 1200 ng g<sup>-1</sup> d.w., with an average and standard deviation of  $56 \pm 160$  ng g<sup>-1</sup> d.w. The concentrations and PCB congener patterns strongly suggest local use as the major explanation of their presence in Cedar Rapids soils: concentrations are ~ 1 order of magnitude higher than background worldwide soil concentrations; and the individual congener profiles observed in the samples are very different from each other and dissimilar to Aroclor mixtures. Overall, the soils are enriched in penta-, hexa- and heptachlorobiphenyls (85%) and exhibit some evidence of environmental weathering from volatilization and/or aerobic biodegradation. Soil toxic equivalency quantities (TEQs) in Cedar Rapids are similar to those reported elsewhere, but 2 order of magnitude lower than a PCB Superfund site. Although the flooding severely affected the industries and residential areas of Cedar Rapids, no spatial trends in PCBs or chlordane was detected that could be attributed to chemical transport during the flood, which has been seen in other similar studies.

**553 Background Concentrations of Dioxin in Urban Soils Throughout the United States: A Review of Academic and Government Literature Values** M.K. Lambert, Univ of Rhode Island, Graduate School of Oceanography, USEPA, OSRTI, Environmental Scientist; M. Berg, USEPA, Office of Superfund Remediation and Technology Innovation. Dioxin is a problematic environmental contaminant due to its persistence, its high toxicity at low concentrations, and its potential to bioaccumulate. Improvements in analytical technologies have lowered the detection limits for measuring dioxin and contributed to an understanding that dioxin is a ubiquitous soil contaminant. An EPA survey of rural soils throughout the US indicated an average rural soil concentration of 1.76 pg TEQ/g (SD = 2.47). Long-range transport, via atmospheric deposition, is the dominant source for dioxin at many of the rural sites studied. Urban background values are more difficult to define due to the proximity of a variety of sources and multiple transport mechanisms. The purpose of this project was to compile published urban background levels of dioxins in soils, provide a national perspective on urban dioxin levels, and highlight important gaps in our knowledge. Much of the data available for the US comes from state funded studies and background studies at individual Superfund sites. Urban residential soil concentrations, as reported by several state funded studies, ranged from 0.1 to 20 pg TEQ/g soil and were similar to values reported for residential soils in other developed countries. Industrial land use soils varied over three orders of magnitude, up to ~1,000 pg TEQ/g.

**554 Metal Contamination in Street Dust: Levels, Trends, and Fate in Urban Watersheds** L.A. Benedict, J. Barilone, N. Chouinard, M. Neidig, Univ of Southern Maine, Chemistry; L. Fox, Univ of Southern Maine; L. Benedict, AMEC. Street dust is defined as fine-grained solids with particle sizes < 500 µm that are found on roadways, parking lots and/or sidewalks. Street dust is predominantly composed of locally derived material (e.g., soils) and is heavily influenced by anthropogenic activities such as driving, construction and demolition, and combustion (e.g., burning). During rain events street dust is transported from impervious surfaces into urban watersheds typically through managed storm water disposal networks. Small-scale urban watersheds are particularly sensitive to contaminated storm water run-off transported from impervious surfaces. Contaminated street dust also poses a human health risk for individuals as the fine-grained particles can easily become airborne and can be inhaled or inadvertently ingested

by adults and children during outdoor activities. This study focused on the determination of levels, fate and trends of various metals found within street dust samples collected from paved areas (e.g., streets and parking lots) in the Casco Bay Watershed in southern Maine. Metal analysis was conducted using an x-ray fluorescence spectrometer. Reported concentrations were evaluated in three sample sets based on the area in which the samples were collected; commercial areas, school areas and residential areas. Average levels of Cu, Zn, Cr, and As were highest in samples collected in commercial areas, likely a result of higher traffic and operations of businesses adjacent to sample sites. Conversely, levels of these four metals were lowest in samples collected from residential areas. Levels of Pb were highest in residential areas, correlating with a higher historical use of lead paint. Samples collected in school areas had levels of Cu, Zn, Cr, and As between those reported for the commercial and residential groups, likely due to the high amount of idling that occurs during drop off and pick up times for schools. This project also focused on the potential of contaminants found in street dust to impact a small-scale urban watershed with a high percentage (28%) of impervious surface and over 90% commercial use. Samples were collected from areas with varying traffic patterns and use. Samples collected from roadways with higher traffic patterns and longer periods of vehicle idling had significantly higher levels of Cu, Zn, and Cr, than those samples collected from areas with little to no idling or with low traffic patterns.

**555 Transport of PCBs, PBDEs, PAHs, and Polycyclic Musks via Urban Tributaries to Lake Ontario, Canada** M. Robson, Univ of Toronto; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; G. Kaltenecker, B. Gilbert, Ontario Ministry of the Environment; L. Melymuk, M. Diamond, Univ of Toronto; C. Brimacombe, T. Chen, T. Kolic, E. Reiner, Ontario Ministry of the Environment. Urban rivers continue to deliver contaminants such as the legacy polychlorinated biphenyls (PCBs) and current/recent-use chemicals to receiving waters like the Great Lakes where they may persist, bioaccumulate, and impact biota. To investigate the relative roles of urban contaminant fate pathways (atmospheric deposition, tributary, municipal wastewater) to adjacent waterbodies, using Toronto, Canada and Lake Ontario as examples, we measured concentrations for a suite of organic compounds of varying uses and sources and estimated stream loadings. This presentation highlights results for the urban tributary pathway. Water concentrations of PCBs, polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenylethers (PBDEs), and polycyclic musks (PCMs) were determined at base flow and in wet weather conditions in Toronto, Ontario, Canada in 2007 through 2009. Median (maximum) concentrations at downstream sites ranged from 2.2-6.7 (19-144) ng/L for SPCBs, 100-950 (1700-19300) ng/L for SPAHs, 5.1-6.6 (25-46) ng/L for SPBDEs, and 29-1800 (53-5800) ng/L for SPCMs. Regression analysis indicated a strong correlation with suspended sediments for PAHs and PCBs ( $R^2$  of 0.59-0.85 and 0.36-0.75, respectively), while correlations were weaker for PBDEs and musks ( $R^2$  of 0.01-0.59 and 0-0.55, respectively), depending on the site. This suggests that the reservoir for PCBs and PAHs are predominantly soils and sediments within the watersheds, while sources are more varied for the current-use PBDEs and musks. Contaminant loads to Lake Ontario were calculated using USGS's LOADest. Estimated average annual loads were 2200 kg/yr for SPAHs, 42 kg/yr for SPCMs, and 8 kg/yr each for SPCBs and SPBDEs. On a mass basis, the tributaries were the most important pathway for SPAHs, contributing 60% of loads compared to atmospheric and wastewater pathways from urban Toronto. In comparison the tributaries were estimated to contribute 40% for SPBDEs, 20% for SPCBs, and 4% for SPCMs. Urban tributaries are important contributors of compounds with urban sources to adjacent aquatic environments.

**556 Predicting Urban Scale Atmospheric PCB and PBDE Emissions and Fate Using the BLFM-MUM Model** S.A. Csiszar, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; S. Daggupati, Environment Canada, Science And Technology Branch; L.E. Melymuk, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; S. Verkoeyen, Univ of Toronto; M. Robson, Univ of Toronto, Dept of Geography; A. Giang, Univ of Toronto; P. Helm, Ontario Ministry of Environment; M.L. Diamond, Univ of Toronto, Dept of Geography and Dept of Chemical Engineering and Applied Chemistry. An atmospheric transport model coupled with a local scale (5×5 km<sup>2</sup>) multi-box multimedia fate model (together referred to as BLFM-MUM) has been developed to estimate chemical emissions and fate in urban areas. The model is illustrated

here through a case study of PCBs and PBDEs in Toronto. Toronto was estimated to contain ~440 tonnes of PCBs in 2006 and ~240 tonnes of PBDEs in 2010. The spatial mass inventories were used to estimate air emissions on a local scale with a total PCB emission of ~150 kg y<sup>-1</sup> or 0.4 g y<sup>-1</sup> kg<sup>-1</sup> inventory for the entire city. These emissions accounted for air concentrations measured at various sampling sites across the city suggesting that neighbouring areas do not significantly contribute to Toronto PCB and PBDE levels. The downtown core had the largest chemical inventories which accounted for ~90% of the total urban PCB emissions. In addition to emissions, local meteorology contributed to concentration gradients measured across the city. The coupled model also simulated interactions between air and the city via various urban media such as soil and films that coat impervious surfaces. The model predicted that a large proportion of chemical becomes diluted within the city's air as modeled air concentrations are at least ~4 times lower at non-downtown sites and are even lower outside the city. The chemical that is not diluted in the air may act as a source to other urban media. We estimate that 45% of the chemical that is deposited to the city is transferred to impervious surface films which is mostly mobilized via storm water.

**557 What We Found in Our Backyard** M.L. Diamond, Univ of Toronto, Dept of Geography and program in planning, Univ of Toronto, Dept of Geography; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; S.A. Csiszar, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; L. Melymuk, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; M. Robson, S. Verkoeyen, Univ of Toronto, Dept of Geography and program in planning; S. Backus, S. Daggupaty, L.M. Jantunen, Environment Canada. We found a lot in our backyard! This presentation summarizes results from our multi-year, multi-agency effort to quantify what's in our backyard and how it moves from emission source in our city to our backyard, urban surface waters and beyond. Focussing on PCBs, PBDEs, PAH and PCMs (polycyclic musks), we measured concentrations in air, soil, urban rivers and final effluent from waste water treatment plants in Toronto. As expected, air concentrations showed strong urban-rural gradients, with the gradient related to patterns of chemical use and thus emissions. For example, Melymuk found that PCB emissions can be explained by major emissions from the downtown core and radial dilution from the core. Robson found a strong correlation between PCM air concentrations and population density. PBDE air concentrations were related to a spatial inventory of PBDEs in Toronto prepared by Verkoeyen. Robson and Helm estimated that loadings from urban rivers were greatest for PAH, followed by PBDEs and PCBs. Concentrations were closely correlated with that of total suspended solids. The greatest loadings of all compounds from rivers occurred during storm events. Emissions from Toronto's waste water treatment plants were greatest for PCMs, followed by PBDEs, PCBs and PAH. Finally, Csiszar and Daggupaty tightly coupled the mesoscale boundary layer forecast meteorology model (BLFMPop) and the Multimedia Urban Model (MUM) which was used to estimate spatially resolved emissions and fate of PCBs and PBDEs. Model results provided a mechanistic explanation for the observations discussed above that were based on the measurement campaign.

**558 Development of Transcriptomics-based Biomarkers for Selected Endocrine Disrupting Chemicals in Zebrafish (*Danio rerio*)** R. Wang, USEPA; D. Bencic, USEPA, Office of Research and Development; A. Biales, USEPA, Office of Research and Development, National Exposure Research Laboratory; R. Flick, USEPA, Office of Research and Development, National Exposure Research Laboratory; J.M. Lazorchak, USEPA, Office of Research and Development, National Exposure Research Laboratory, Molecular Ecology Research Branch; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division. Genome-wide transcriptional profiling by microarrays provides a powerful platform for gene expression-based biomarker discovery. After their wide acceptance in human disease diagnosis, prognosis, and drug discovery, these gene signatures are increasingly being adopted for environmental toxicology applications as well. Built upon our previous work in zebrafish of reverse-engineered transcription factor (TF) networks and their statistical linkage to various treatment conditions of endocrine disrupting chemicals (EDCs), a systematic, computationally-intensive search using genetic algorithm coupled with support vector machine was conducted for candidate gene signatures capable of distinguishing

individual chemical treatment conditions from their controls. Microarray data from zebrafish brain (male, female, or combined), ovary, and testis were analyzed. The search within each tissue type was either confined to hundreds of individual TF networks significantly associated with EDCs of differing mechanisms/modes of action, or across the entire genome. In agreement with our previous findings, tissue type was found to be critical to biomarker search. Brain yielded most candidate gene signatures, followed by ovary and testis. By chemical, prochloraz had the greatest number of candidates, then flutamide, fipronil, and ethinyl estradiol. A subset of these candidates will be evaluated using an independently derived microarray dataset generated from additional EDC-exposed zebrafish samples.

**559 Transcriptomic Responses to Metal Bioaccumulation in the Blue Mussel *Mytilus edulis*** H.C. Poynton, US Environmental Protection Agency, Molecular Indicators Research, Univ of Massachusetts-Boston, Environmental, Earth and Ocean Sciences; W.E. Robinson, Univ of Massachusetts-Boston, EEOS Dept; B.J. Blalock, Univ of Massachusetts, Boston, Environmental, Earth, and Ocean Sciences; R.E. Hannigan, Univ of Massachusetts, Boston, Dept of Environmental, Earth and Ocean Sciences. In the Northeastern United States, the blue mussel *Mytilus edulis* is the primary species utilized for monitoring the bioaccumulation of toxicants along the coast. The NOAA National Status and Trends "Mussel Watch" Program has monitored tissue concentrations of contaminants in this species for over thirty years; however, as the list of potential contaminants grows, the ability to measure concentrations in a targeted manner is becoming cost prohibitive. Bioassays which are able to screen for pollution and predict the presence of chemical contaminants are needed. We created a 3500 gene *M. edulis* oligonucleotide microarray to determine gene expression patterns that correlate with the bioaccumulation of cadmium and lead. We performed four week exposures to 0.54 µM Cd and 0.54 µM Pb and a mixture of both metals and monitored gene expression in the gill after one week, two weeks and four weeks. Metal bioaccumulation was also measured in the gill, digestive gland and whole body soft tissue at these time points. Transcriptomic patterns after two weeks reveal distinct expression profiles for the two metals with the mixture profile most closely resembling the Cd profile, possibly due to competition for uptake between the two metals or increased toxicity of Cd. As expected, metallothionein biomarker genes MT-10 and MT-20 were induced in the Cd and mixture exposures confirming the validity of the transcriptomic approach. Analysis of the one week and four week time points will enable us to determine if gene expression profiles of more chronically exposed organisms directly correspond to the levels of metals accumulated in their tissues.

**560 Epigenetic Effects of Methylmercury on DNA Methylation in Fish, Mammals, and Birds** J. Head, Univ of Michigan, Cooperative Institute of Limnology and Ecosystem Research, Univ of Michigan, Cooperative Institute for Limnology and Ecosystem Research; D. Nam, Univ of Michigan, Dept of Environmental Health Sciences; J. Rutkiewicz, Univ of Michigan; K. Mittal, Univ of Michigan, Dept of Environmental Health Sciences; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. We are interested in studying the epigenetic effects of environmental contaminants in natural populations. Alterations in patterns of DNA methylation are termed 'epigenetic' because they cause heritable changes to gene expression that occur outside of changes to the DNA sequence itself. We previously reported that relatively low levels of methylmercury are associated with a decrease in global DNA methylation in mammalian brain tissue. This decrease was observed in wild polar bears and captive mink, and was accompanied by a suppression of DNA methyltransferase (DNMT), the enzyme that maintains DNA methylation. Here we expand upon this work by assessing the relationship between methylmercury exposure and global DNA methylation in multiple tissue types (liver, muscle, brain) and species (mammals, fish and birds). Global DNA methylation ranged from 53% in chicken to 87% in yellow perch. No significant differences in DNA methylation were observed among tissue types for a given species. The effect of methylmercury on DNA methylation may be limited to certain species or tissues. For example, no effect of methylmercury was detected in yellow perch liver or muscle. Ongoing work will characterize the effects of methylmercury on DNA methylation in chicken tissues. By taking a multi-species, multi-tissue approach we hope to add weight of evidence to our initial findings and also gain a deeper understanding of the mechanisms behind contaminant-induced alterations to DNA methylation across ecologically-relevant organisms.



**562 Identification and Characterization of Reptilian Cytochrome P450 1A: Sequencing, Expression, and Inducibility of CYP1A5 in Sea Turtles** C. Godard-Codding, The Institute of Environmental and Human Health/ Texas Tech Univ, Assistant Professor, Texas Tech Univ/The Institute of Environmental and Human Health, Assistant Professor; S. Wiggins, Texas Tech Univ/The Institute of Environmental and Human Health; J. Goldstone, Woods Hole Oceanographic Institution; J. Flanagan, Houston Zoo; B. Higgins, NOAA/NMFS. The loggerhead sea turtle (*Caretta caretta*) is currently listed as threatened under the Endangered Species Act. It faces threats from anthropogenic sources including exposure to environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs). The metabolism and toxicity of these and other xenobiotics are poorly known in this species and in reptiles in general. This knowledge gap limits our understanding of the impact of environmental pollution on the health and survival of sea turtles and thus our ability to conserve and manage them. The cytochrome P450 1A1 (CYP1A1) enzyme plays a key role in the metabolism and activation of PAHs to their carcinogenic metabolites in mammals and may be involved in the bioactivation of PAHs in reptiles as well. A CYP1A protein has been detected in some reptiles; however, the full length CYP1A gene has not yet been sequenced in turtles or any other reptilian species to the best of our knowledge. Clones isolated from the liver of three species, the loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*) and green sea turtles (*Chelonia mydas*), were identified and named as CYP1A5 by the P450 Nomenclature Committee. Reptilian CYP1A5 share between 58-67% amino acid sequence identity with mammalian and amphibian CYP1As and between 65-79% amino acid sequence identity with avian CYP1As. Tissue distribution and expression of CYP1A5 was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in all loggerhead tissue types tested (heart, kidney, liver, lung, muscle, ovary, and skin). CYP1A1 and CYP1A-like induction is commonly used as a biomarker of exposure to PAHs and PHAHs in wildlife, but its relevance in sea turtles and other reptiles has not yet been validated. We investigated the CYP1A5 inducibility at the gene expression level using primary skin fibroblast cultures established in our laboratory. The cells were exposed to benzo[a]pyrene (B[a]P) for 72 hours and CYP1A5 induction by this prototypical PAH was detected by qRT-PCR. The present study confirms that CYP1A exists in reptiles and that sea turtle CYP1A5 is inducible by a xenobiotic known to induce CYP1A in other species.

**564 The Effect of CYP1A Inhibition on Embryotoxicity of Weak Aryl Hydrocarbon Receptor Agonists in *Fundulus heteroclitus* and *Danio rerio*** D. Brown, B. Clark, L. Van Tiem, Duke Univ, Nicholas School of the Environment; K. Johnson, Duke Univ, Howard Hughes Program; R. Di Giulio, Duke Univ, Nicholas School of the Environment. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcriptional regulator that mediates many of the toxic effects of dioxin-like compounds and many polycyclic aromatic hydrocarbons (PAHs). Strong AHR agonists, such as some polychlorinated biphenyls and dioxins, cause severe cardiac teratogenesis in fish embryos. We have shown that moderately strong PAH agonists such as benzo[a]pyrene and b-naphthoflavone cause similar cardiac teratogenesis when coupled with a CYP1A inhibitor such as fluoranthene (FL). We sought to determine if weaker agonists are capable of causing cardiac deformities following inhibition of CYP1A with FL. *Fundulus heteroclitus* (killifish) and *Danio rerio* (zebrafish) embryos were exposed to weak AHR agonists (carbaryl, phenanthrene, 2-methylindole, 3-methylindole, indigo, and indirubin) at equimolar concentrations (100 nM and 1 µM). The agonists were assessed for their relative potency as inducers of CYP1 and capacity for inducing cardiac teratogenesis. CYP1 induction was measured via the in vivo ethoxyresorufin-O-deethylase assay at 96 hpf. Indirubin, Indigo, and carbaryl were the strongest CYP1 inducers in killifish embryos showing significant differences from the control treatment. In contrast, 3-methylindole, carbaryl, and 2-methylindole caused the highest CYP1 activity in zebrafish. These equimolar exposures did not cause deformities. Agonists were then coupled with FL to assess if CYP1A inhibition could induce cardiac deformities. Killifish cardiac deformities were assessed at 144 hpf, and zebrafish cardiac deformities were measured at 96 hpf. FL by itself did not cause deformities, but co-exposure with some weak agonists resulted in cardiac teratogenesis. Cardiac deformities were significantly increased in the indigo + FL and indirubin + FL groups in killifish embryos, whereas FL enhanced the teratogenicity of 2-methylindole, carbaryl, phenanthrene, and 3-methylindole in zebrafish. Current studies are employing knockdown of

AHR2 in both species. AHR2 has been shown previously to mediate the embryotoxicity of weak and moderate agonists. P42-ES10356.

**565 Modulation of Lipid Levels and Cellular Homeostatic and Apoptotic Responses in Salmon Hepatocytes is a Novel Aspect of PFOSA Toxicity** A.M. Wagbo, Norwegian Univ of Science and Technology (NTNU), Biology; M.V. Cangialosi, Univ of Messina; R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environment, Canadian Wildlife Service; G. Dugo, Univ of Messina, Biology; A. Arukwe, Norwegian Univ of Science and Technology (NTNU), Dept of Biology, Norwegian Univ of Science and Technology (NTNU), Biology. Available toxicological evidence indicates that perfluorinated organic compounds (PFCs) alter normal peroxisomal lipid oxidation and cellular function in mammalian in vivo and in vitro studies. The neutral perfluorooctane sulfonamide (PFOSA) has received little attention over the years, but recent evidence show that PFOSA can be metabolically degraded at a slow rate and is a potent mediator of oxidative stress and cell death. The primary objective of the present study was to investigate the effects of PFOSA on cellular functions and lipid homeostasis (including  $\beta$ -oxidation) in salmon in vitro primary hepatocyte system. Primary salmon hepatocytes generated by two-step perfusion technique were exposed to PFOSA at 2, 20 and 50 µM for 0, 12 and 24 h. Fatty acids (FAs) and lipids were determined by GC-MS, fatty acid elongase (FAE), desaturase-5 (FAD5) and desaturase-6 (FAD6) and PPARs (a, b and g) and acyl coenzyme A (ACOX-1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), autophagy gene (ATG) 5, beclin-1 and bcl-2 19 kD interacting protein 3 (bnip3) mRNA expression were analyzed using qPCR. Our data showed that PFOSA produced a time- and concentration-dependent significant alteration of FA profiles. PFOSA produced changes in FA composition that predominantly involved decrease (at 24 h) and increase (at 48 h) in fatty acid methyl esters (FAMES), MUFA, total PUFA, (n-3 and n-6) PUFA. Particularly, an increase of g and a-linolenic acid [ALA; 18:3(n-6 and n-3)], eicosapentaenoic acid [EPA; 20:5(n-3)] and arachidonic acid (ARA; 20:4 n6) with associated increase in FAE, FAD5 and FAD6 mRNA expression were observed after PFOSA exposure, while docosadienoic acid [DCA; 22:2(n-6)] was significantly decreased. PFOSA produced apparent concentration-dependent increase of PPAR $\alpha$  and PPAR $\gamma$ , while PPAR $\beta$  was not affected. The level of GAPDH mRNA declined at 24 h. ATG-5 was not significantly affected, while at 12h exposure, the levels of beclin-1 was increased and decreased at 20 and 50 µM, respectively. Our data also showed that PFOSA produced an increase in the levels of bnip3. In general, these responses indicate that PFOSA evoke deleterious effect on cellular lipid homeostasis and  $\beta$ -oxidation and responses that regulate cellular homeostasis and apoptosis in salmon hepatocytes.

**566 Evaluation of the Immunological Effects of Oil Sands-influenced Surface Waters Using a Rainbow Trout In Situ Bioassay** S.A. McNeill, N.S. Hogan, Canadian Rivers Institute, Univ of Prince Edward Island, Dept of Biology; C.J. Arens, M.R. Vandenhevel, Canadian Rivers Institute, Univ of Prince Edward Island. The Athabasca oil sands deposit located in northeastern Alberta is the largest single oil reserve in the world, containing over 700 billion barrels of bitumen. The reclamation of the mined area will introduce new aquatic habitat that may be influenced by organic and inorganic compounds associated with oil sands. A 21 day in situ bioassay was conducted with rainbow trout (*Oncorhynchus mykiss*) co-exposed to oil sands-influenced surface waters and pathogen-associated molecular patterns in the form of inactivated *Aeromonas salmonicida* (A.s.). Rainbow trout were stocked in 1 m<sup>3</sup> constructed cages at three sites: (1) Mildred Lake, a reservoir receiving only Athabasca River water, used as the reference site; (2) South Bison Pond, a drainage basin that has received unextracted oil sands-contaminated overburden; and (3) Demonstration Pond, a constructed pond containing oil sands mature fine tailings capped with fresh water. After acclimation, trout were tagged and injected with either phosphate buffered saline (control) or formalin-inactivated A.s. Trout were sacrificed and processed 21 days post-injection. Some mortality was observed in rainbow trout caged in the Demonstration Pond and opacity of the eyes indicated that this may have been due to gas bubble formation as the pond is consistently gas supersaturated. Rainbow trout from the Demonstration Pond showed reduced total leukocytes as compared to the other two sites. The A.s. treatment itself caused an overall elevation of leukocytes. Demonstration Pond fish also showed increased fin erosion as evaluated by a fin erosion index. There were

no significant differences in liver weight, spleen weight or condition factor across the treatments. Results will be discussed with regards to fish exposure as measured by water chemistry, bile fluorescent metabolites, and liver monooxygenase activity.

**567 Long-term Changes in Disease Incidence and Biochemical Indicators of Exposure in Yellow Perch (*Perca flavescens*) Exposed to Oil Sands-Affected Waters** N.S. Hogan, Canadian Rivers Institute, Univ of Prince Edward Island, Dept of Biology; L.J. Phalen, M.R. van den Heuvel, Univ of Prince Edward Island, Dept of Biology. Large quantities of affected water and solid materials from oil sands mining in northern Alberta, Canada must eventually be incorporated into the reclaimed landscape. This material contains a mixture of organic and inorganic compounds, specifically elevated salt levels, naphthenic acids and traces of polycyclic aromatic hydrocarbons (PAHs). To examine the potential effects of oil sands-influenced aquatic systems on fish health, studies were conducted during 1995-1997 and 2008-2010 whereby yellow perch were captured from a nearby reservoir, Mildred Lake, and placed two experimental ponds: South Bison Pond, a drainage basin that has received unextracted oil sands-contaminated overburden or Demonstration Pond, a constructed pond containing oil sands mature fine tailings capped with fresh water. Liver mixed-function oxygenase activity as measured by 7-ethoxoresorufin-O-deethylase (EROD) activity was highest in fish from experimental ponds and this pattern was consistent across all sampling years. Bile fluorescence measurements showed a gradient of oil sands-associated exposure with the two experimental ponds being elevated over the source population of perch, and over other local perch populations. In both sets of studies, two disease pathologies were observed in yellow perch: fin erosion caused by an as yet unidentified pathogen, and lymphocystis that was confirmed using a diagnostic real-time PCR method. In early studies (1995-1997) both pathologies were most prevalent in the South Bison Pond; however, in the recent studies (2008-2009), disease was substantially more prevalent in the Demonstration Pond and corresponded with a decrease in total white blood cells. Changes in pond chemistry over the past decade allow for some inferences to be made as to causal agents. Naphthenic acids have decreased in the Bison Pond over the duration of studies while increasing in the Demonstration Pond due to tailings densification. As the concentrations of inorganic compounds in the ponds are similar, temporal changes in naphthenic acid levels appear to correlate best with incidence of disease pathologies, an effect that may be related to a contaminant-induced immune suppression. However, the precise chemical makeup of the organic material, including the identity of the fluorescent bile metabolites is not yet fully understood. The causative agents and their thresholds for immunological effects will dictate further research directions.

**568 Toxicity of Untreated and Ozone-treated Oil Sands Process-Affected Water (OSPW) to Early Life Stages of the Fathead Minnow (*Pimephales promelas*)** Y. He, Univ of Saskatchewan, Toxicology Centre, Univ of Saskatchewan, PHD student; S.B. Wiseman, M. Hecker, Univ of Saskatchewan, Toxicology Centre; P. Jones, Univ of Saskatchewan, School of Environment and Sustainability; A. Moustafa, N. Wang, L. Perez, Univ of Alberta, Dept of Civil and Environmental Engineering; M.G. Din, Univ of Alberta, Dept of Civil and Environmental Engineering; J.W. Martin, Univ of Alberta, Laboratory Medicine and Pathology, Univ of Alberta, Dept of Lab Medicine and Pathology, Univ of Alberta, Dept of Public Health Science; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science. Great volumes of oil sands process-affected water (OSPW) are produced annually by the oil sands industry in North Eastern Alberta, Canada. OSPW is stored in on-site tailings ponds due to a no-release policy implemented as the result of concerns about the potential toxicity and remediation of this process water. Ozonation may be a promising and effective method for remediation of OSPW, by reducing concentration of naphthenic acids, which are considered the primary toxic constituents in OSPW. However, further work is needed to fully evaluate the effectiveness of ozonation in reducing the toxicity of OSPW and to ensure that ozonation does not impart toxicity upon OSPW. This study examined the effects of untreated and ozone-treated OSPW on the early life stage (ELS) of fathead minnow (*Pimephales promelas*). Embryo mortality rates, spontaneous embryo movement, hatching success, and incidences of hemorrhage, pericardial edema, and spinal malformation were examined. Exposure to OSPW significantly increased the incidence of embryo mortality ( $56 \pm 14\%$ ), compared to that for control and ozone-treated OSPW ( $2.1 \pm 4.2\%$  and  $23 \pm 14\%$ , respectively) ( $p <$

$0.05$ , the same below). Exposure to OSPW also significantly increased the rate of spontaneous embryo movement at 26 hpf ( $49 \pm 5.5$  movements/min), compared to  $25 \pm 2.3$  movements/min in control and  $29 \pm 3.8$  movements/min in ozone-treated OSPW. Exposure to OSPW resulted in a greater incidence of immature hatching, with the cumulative hatching rates of  $15 \pm 4.2\%$ ,  $31 \pm 8.0\%$ , and  $69 \pm 8.0\%$  at 48, 72, and 96 hpf, respectively, while at the same time points the cumulative hatching rates were  $2.1 \pm 4.2\%$ ,  $8.3 \pm 12\%$ , and  $29\% \pm 20\%$  in ozone-treated group. No embryos hatched until 120 hpf in control. Finally, exposure to OSPW also resulted in greater incidences of hemorrhage ( $50 \pm 6.8\%$ ), pericardial edema ( $56 \pm 14\%$ ) and spinal malformation ( $38 \pm 11\%$ ), compared to ozone-treated group ( $13 \pm 4.8\%$ ,  $6.3 \pm 8.0\%$ , and  $6.3 \pm 4.2\%$ ). No malformations were observed in control. Overall, the results suggest that exposure to OSPW can negatively impact early embryo development of the fathead minnow, and ozonation of OSPW attenuated the observed toxicity towards ELS of fathead minnow. The mechanisms of the observed effects of OSPW are currently under investigation and will be discussed.

**569 Using Synchronous Fluorescence Spectroscopy to Prioritize Environmental Samples for High Resolution Mass Spectrometry in Canada's Oil Sands** R.A. Frank, Environment Canada, AEPRD, Univ of Guelph, Dept of Env. Biology & Toxicology; G. Bickerton, J. Headley, M. McMaster, M. Hewitt, Environment Canada. The oil sands of northern Alberta, Canada contain an estimated 170 billion barrels of recoverable crude oil, second only to Saudi Arabia's 264 billion barrels. These proven Alberta reserves only account for approximately 10% of the total crude contained within the oil sands deposits and as technology improves, future production possibilities could make Canada the world leader in oil supply. However, the rapid expansion of oil sands development, especially within the past 10 years, has raised many concerns regarding the potential impacts on the environment and communities downstream of oil sands development. Extensive research is currently underway to identify a "chemical fingerprint" capable of differentiating oil sands materials originating from natural sources of bitumen from industrial sources, such as leachate from tailings ponds, entering surface waters. Differentiation of oil sands materials is critical to both industry and regulators alike, as accurate monitoring of potential pollution is currently quite difficult. Recent advancements in high resolution mass spectrometry analyses are beginning to allow for the elucidation of specific classes of compounds as well as some individual structures contained within complex mixtures of oil sands organic acids. Due to the high costs associated with high resolution mass spectrometry, a screening method using synchronous fluorescence spectroscopy (SFS) to prioritize samples for further analysis was developed. Following the identification of a unique SFS fingerprint for oil sands organic acids, a large number of samples collected from the Athabasca River and its tributaries, basins containing oil sands process-affected water (OSPW), groundwater interceptor wells near basins containing OSPW, and groundwater wells and seeps along the banks of the Athabasca River and its tributaries have been screened. This enabled the identification of specific classes of compounds as fingerprinting candidates for determining tailings seepage using high resolution mass spectrometry.

**570 Identification and Toxicity of Individual Naphthenic Acids** S.J. Rowland, Univ of Plymouth, School of Geography, Earth and Environmental Sciences, Univ of Plymouth, SoGEES; C. West, Univ of Plymouth, SoGEES; A. Scarlett, Univ of Plymouth, School of Earth, Ocean & Environmental Science; D. Jones, Univ of Plymouth, School of Geography, Earth&Environm. Science. Schindler<sup>1</sup> has called for the debate on the environmental consequences of oil sands exploitation in Canada to be based on solid science. Kean<sup>2</sup> has labelled aspects of the current debate, "eco-alchemy". Although the acute impacts of oil sands process water have sometimes been dramatic, experimental evidence for the acute toxicity of the so-called 'naphthenic acids' has been mainly gathered from assays of incompletely characterised extracts of the water. Clearly the toxic constituents need to be identified and assayed. We show how numerous of these acids in oil sands process water and petroleum-derived naphthenic acids can be identified by comprehensive two-dimensional gas chromatography-mass spectrometry. We then show how synthesis of dozens of such acyclic to tricyclic acids and measurement of their toxicities to the bioluminescent bacterium, *Vibrio fischeri* allows assessment of their toxic effects. We also used a computer model to predict their toxicities to the water flea, *Daphnia magna*. Both are well-accepted toxicological screening endpoints. The results show how toxic some of the naphthenic acids really are and reveal the influence of hydrophobicity



and aqueous solubility on the toxicities. Comparison with measured toxicities of other known, but more minor, constituents of oil sands process water, such as polycyclic aromatic hydrocarbons and alkylphenols, helps place these toxicities into a wider context. 1. Schindler, D. Tar sands needs solid science. *Nature* 2010, 468, 499-501. 1. 2. Kean, S. Eco-alchemy in Alberta. *Science* 2009, 326, 1052-1055.

**571 Tracking the Sources of Naphthenic Acids in the Athabasca River and its Tributaries** J.W. Martin, Univ of Alberta, Laboratory Medicine and Pathology, Univ of Alberta, Dept of Lab Medicine and Pathology, Univ of Alberta, Dept of Public Health Science; J.P. Benskin, AXYS Analytical Services Ltd; M.S. Ross, Univ of Alberta, Dept of Laboratory Medicine & Pathology; E. King, Univ of Alberta. Highly specific and sensitive analytical methods have recently evolved for accurately characterizing the complex mixture of organic acids in oil sands process water (OSPW), including naphthenic acids (NAs). Here we report the application of HPLC-high resolution mass spectrometry to background water samples in the oil sands region. Samples (1 L) were collected in Fall 2010 at sites upstream and downstream of industrial activity and were preserved with methanol, and by freezing. Sampling locations included the main Athabasca River, upstream and downstream of industrial activity, and also smaller tributaries in the open-pit mining region. The North Saskatchewan River was also sampled, in Edmonton, for comparison. Samples were extracted by acidification and liquid-liquid extraction using an isotopically labeled internal standard for quantification. NA profiles in samples close to human development (i.e., Fort McMurray, or Edmonton) had a prevalence of non-cyclic ( $Z=0$ ) NAs, presumably owing to emissions of surfactants from municipal waste-water treatment plants. Concentrations in the main stem of the Athabasca River were low, immediately upstream of industrial activity, but NAs were detectable downstream at several sites. The upstream headwaters of small tributaries to the Athabasca River had very low NA concentrations, but at the mouth of these rivers (where they join the Athabasca River) NA concentrations were detectable. NA profiles of natural water samples were compared to OSPW, and data will be discussed with respect to differentiating natural sources of NAs from anthropogenic emissions of OSPW.

**572 A Comparison Between Carboxyl Groups Quantifications for Different Naphthenic Acids Using the Linear Programming Technique and the FT-IR Spectroscopy** A.M. Moustafa, Univ of Alberta, Dept of Civil and Environmental Engineering; S. Kang, Kyung Hee Univ, Dept of Civil Engineering; S. Smith, Wilfrid Laurier Univ, Faculty of Science; J. Wang, M.M. Gamal El-Din, Univ of Alberta, Dept of Civil and Environmental Engineering. Naphthenic acids (NAs) are the major toxic compounds from the bitumen extraction industries in northern Alberta. In NAs, carboxylic functional groups are attached to unspecified alicyclic compounds with different molecular weights. The FT-IR spectroscopy is one of the popular quantification method for NAs by the detection of the carboxylic acids through their carbonyl stretch  $C=O$ . Also this quantification is generally used for the assessment of the solution toxicity. The purpose of the current study is to quantify the actual concentrations of carboxylic functional groups for different sources of NAs samples. The quantification techniques performed using the Linear Programming Method (LPM) and compared to the FT-IR results. 50 mg/L NAs solutions were prepared from the refined Merichem NAs, the Fluka NAs, the OSPW-extracted NAs and the Cyclohexanecarboxylic acid. All concentrations were confirmed using the FT-IR spectroscopy, but only 40 mg/L of the Cyclohexanecarboxylic acid was detected. The evaluation of their toxicity was performed using the basic protocol of the Microtox bioassay. The concentration of the carboxylic functional groups for all NAs sources were secondly evaluated using the Linear Programming Method (Cox et al. 1999). Results showed that both Merichem and Fluka NAs have almost the same concentration of carboxylic functional groups  $3.3 \times 10^{-4}$  mM, and the same toxicity as well. The extracted NAs from OSPW showed only  $1.15 \times 10^{-4}$  mM of carboxylic functional groups concentration which equal to one third of Merichem's and Fluka's. Toxicity of NAs from OSPW was 50% of Fluka and Merichem. On the other hand, the carboxylic functional groups concentration for the Cyclohexanecarboxylic acid was  $3.92 \times 10^{-4}$  mM, which is equal to the prepared concentration with no toxicity at all. This study introduced LPM as an efficient method for NAs quantification. LPM technique detects carboxylic groups directly instead of carbonyl groups in the FT-IR method. Also it shows the independency of the toxicity on the total NAs concentration.

**573 Dropping Acid at the Synchrotron: Using X-Ray Absorption Spectroscopy to Characterize Naphthenic Acids** C.H. Ryan, Canadian Light Source Inc.; N. Toor, Level Science Inc., Environmental Science; M. Gillis, Level Science Inc.; L. Zuin, Y. Hu, Canadian Light Source Inc., Experimental Facilities Division; J. Headley, Environment Canada, Water Science and Technology Directorate. The characterization of oil sands acid-extractable organics (AEO), commonly referred to as naphthenic acids (NAs), is a topic of growing interest for the study of their fate and transport in aquatic environments. The principal toxic components in oil sands process-affected waters (OSPW) have not been identified. Reported here is the first account of the application of X-ray absorption near-edge spectroscopy (XANES) for the characterization of the components present within NAs derived from OSPW and surrounding waters. These components are known to interfere with the characterization of NAs using conventional techniques. Emphasis is given to the identification of sulfur components as potential markers for delineating anthropogenic sources of NAs from those that are naturally occurring. The presence of sulfur in different chemical states in OSPW NAs and surrounding waters was confirmed using complimentary beamlines for the sulfur K and L-edges XANES. The qualitative speciation of sulfur suggests large contributions of sulfates, and additional contributions from organosulfur compounds similar to thioethers, benzothiophenes, and sulfoxides. The OSPW NAs XANES were also compared to the spectra obtained from NAs extracted from surrounding waters; upstream, midstream and downstream of oil sands production. A spectral shift was identified in the extracted NAs from upstream to downstream. It suggests a larger organic contribution from the sulfur components within the NAs, as the sample moves downstream.

**574 En Route to Developing a Female Reproductive Assessment Scheme for Mammals in the Field** L. Tannenbaum, US Army Public Health Command, Environmental Health Risk Assessment Program, US Army Institute of Public Health. Direct (reproductive) health status assessment of ecological receptors (mammals) at contaminated sites formally entered the ecological risk assessment (ERA) arena just over a year ago with the Army's patenting of Rodent Sperm Analysis – a method presented at SETAC N.A. meetings in recent years. With reproduction understood by many to be the most sensitive toxicological endpoint for mammals, and arguably being the greatest endpoint of concern in ERA in an overall sense, the need to embellish field-based reproductive assessment is keen. The direct (reproductive) health status assessment approach can be made stronger with the addition of a complementary assessment scheme for female rodents. To that end, a strategy for bringing such a scheme online has been developed. Come hear why "Wild Rodent Ovarian Follicle Counting" ("WROF-C") may well be the way to go. Preliminary WROF-C data might be informing us of a useful and informative threshold-for-effect to apply.

**575 Estimating the Potential Significance of Dermal Exposure of Birds, Reptiles, Amphibians, and Mammals to Pesticides** T. Crk, E. Odenkirchen, US Environmental Protection Agency, Office of Pesticide Programs, Environmental Fate and Effects Division; T. Purucker, US Environmental Protection Agency, Office of Research and Development; D. Spatz, N. Mastrotta, K. Garber, J. Housenger, F. Farruggia, M. Wagman, M. Clock-Rust, V. Woodard, T. Downen, H. Yingling, US Environmental Protection Agency, Office of Pesticide Programs, Environmental Fate and Effects Division. The Dermal Uptake Screening Tool (DUST v. 1.0) is a model developed by the United States Environmental Protection Agency's Office of Pesticide Programs. The output of the model will be used in the problem formulation phase of ecological risk assessment to assess the potential significance of chemical exposure to the dermis of birds, reptiles, amphibians, and mammals as a result of pesticide application. Exposure estimates are based on pesticide application rate, application method, and biology of the non-target organism. DUST evaluates dermal exposures via contact with soil and foliar residues and interception of spray. Soil exposure estimates for terrestrial wildlife account for contact with soil particulates via dust bathing and treading the soil surface. Dermal contact estimates vary across terrestrial taxa more than for other exposure pathways, for example, amphibian dermal contact from enhanced skin permeability can be a key exposure pathway due to high dermis-soil water uptake rates that allow amphibians to regulate hydration. DUST output is used to establish a rationale in problem formulation to either dismiss dermal exposure concerns for soil residues and other dermal contact routes or support further consideration in data collection and analysis phases of the ecological risk assessment. This presentation



includes the following elements of the model: exposure estimation, preliminary effects endpoint estimation, integration of exposure and effects, decision criteria, model assumptions, and model evaluation.

**576 Calculation of Lead Site-specific Soil and Food Ingestion Guideline for Livestock Receptors** J. Widmeyer, EBA A Tetra Tech Company, Ecological Risk Assessment; K. Bessie, EBA, A Tetra Tech Company, Soil Science; R.N. Hull, Intrinsik Environmental Sciences Inc. In July 2010, an Environmental Summary Report (ESR) was completed for the Valleyview Land Treatment Facility. Soil lead concentrations exceeded the current Alberta Tier 2 Guideline (70 mg/kg) for the Livestock Soil and Food Ingestion exposure pathway. Considering that the current CCME lead exposure guideline was derived using an avian receptor, quail, it is not only practical but relevant to derive a site-specific guideline for lead for livestock species actually using the site. For the Valleyview Land Treatment Facility there are three herbivorous receptors included in the Soil Quality Guideline (SQG) recalculation: beef cattle, dairy cattle, and domestic chickens. The following information was used to develop a site-specific lead SQG for the ingestion pathway for livestock: Literature-derived exposure parameters including body weight, food ingestion rate, and incidental soil ingestion rate for each receptor; Correlation of plant lead concentrations with soil concentrations to derive a site-specific BCF, and comparison of the site-specific BCF with a literature-derived BCF (using a regression equation); Derivation of 95% UCLM and 80% percentile soil lead concentrations for both ecologically-relevant soil lead samples (upper 20 cm horizon) and all samples (surface to 80 cm depth); ED for livestock receptors – based upon studies with chronic exposure demonstrating sublethal effects; Application of 100% bioavailability for lead in soil; 100% receptor exposure from dietary uptake of onsite soil and vegetation. SQG for receptors were above soil lead maximum, 95% UCLM, and 80% percentile concentrations, whether compared to lead soil concentrations collected from all depths or from the most biologically-relevant depth onsite (top 20 cm). Chicken exposures are driving the SQG; however, the chicken SQG is 9- to 11-fold above the 80% percentile soil lead concentration. Based upon the recalculated SQG, no unacceptable risks exist for beef cattle, dairy cattle, and domestic chickens ingesting soils and vegetation.

**577 Development of a Soil Toxicity Test Using Species Native to the Canadian Boreal Wetlands** M. Moody, Saskatchewan Research Council, Environment Division; R. Scroggins, Environment Canada, Ecotoxicology and Wildlife Health Division. Development of a toxicity test specific to wetland soils of the boreal eco-zone is a priority recognized by governments and legislators in Canada. Thousands of industrial sites, derived particularly from oil and gas production are found in the boreal wetlands. Therefore, tools are needed to demonstrate impacts of produced water spills containing high levels of salts and hydrocarbons. Candidate test species native to three wetland habitats (bogs, fens and shallow water marshes) were chosen to offer an ecologically relevant test battery. Achieving consistent germination and growth in preliminary tests led to adoption of suitable test durations and measurements of growth in soils and peats collected from clean reference sites. Challenges presented by varying nutrient levels in commercial peats and testing in processed or dried substrates will contribute to development of a test method capable of reflecting impacts in vulnerable wetland habitats.

**578 A Comparative Study of Multiple Drilling Fluids and Their Relative Terrestrial Toxicity** L.K. Arneson, Texas Tech Univ, Dept of Environmental Toxicology, The Institute of Environmental and Human Health (TIEHH), Dept of Environmental Toxicology; D.L. Carr, Texas Tech Univ, Biological Sciences; S. Armstrong, Shell Health – Americas; T. Anderson, Texas Tech Univ, TIEHH, Environmental Toxicology. This study compared the terrestrial toxicity and relative environmental safety of several hydrocarbon-based products developed for land-based drilling. During the drilling process the surrounding terrestrial area can be exposed to drilling fluids as well as the remnants of the drilling process including land disposed drill-cuttings. Comparisons were made between two newly developed gas to liquid (GTL) products and two conventional drilling fluids including a low toxicity mineral oil (LTMO) and a low sulfur diesel (LSD). Acute toxicity tests were performed over a period of 14 days for the earthworm (*E. fetida*) and for germination of alfalfa (*M. sativa*) and a wheatgrass species (*E. lanceolatus*). A saltbush (*A. canescens*) 21-day germination study was also performed. Additionally, a springtail (*F. candida*) 21-day exposure study was performed. GCMS was used to monitor hydrocarbon content in soils.

A G-Test of the earthworm results showed that there was a treatment affect ( $p < 0.001$ ,  $p < 0.001$ , and  $p = 0.008$ ) for freshly spiked, 10° C, and 30° C aged soils. Alfalfa showed no significant difference in germination among drilling fluid treatments. However, results also showed that wheatgrass germination was affected by drilling fluid treatments. Results showed p-values at  $p < 0.0001$ ,  $p = 0.0003$ , and  $p = 0.77$  for freshly spiked, 10° C, and 30° C aged soils, respectively. Overall, the GTL C10-C22 product showed the least terrestrial toxicity, both the LTMO and GTL C11-C24 showed some toxicity although LTMO was highly toxic to earthworms and wheatgrass germination, and Diesel-treated soils showed the most toxic responses.

**579 Cytotoxicity and Interaction of Silver Nanoparticles in *Eisenia andrei*** J. Kwak, Konkuk Univ; W. Lee, Konkuk Univ, Konkuk Univ, Dept of Environmental Science; S. Kim, Konkuk Univ; Y. An, Konkuk Univ, Dept of Environmental Science. The cytotoxicity of citrate-coated silver nanoparticles (cAgNPs) and the changes of cAgNPs in biological fluid using *Eisenia andrei* were investigated. The cytotoxicity was measured using the neutral red retention assay (NRR assay), along with mortality, abnormality, and bioaccumulation. Because cAgNPs were adsorbed and aggregated in soil, the survival and abnormal effects of cAgNPs on *E. andrei* was negligible and low bioaccumulation factors were calculated up to the cAgNPs of 2000 µg/g. However, 7d-NOEC values (lysosomal stability) were 300 µg/g dry soil as a result of NRR assay. Additionally, the effects of silver ions dissolved during exposure duration were not observed. We also dispersed cAgNPs in deionized water, earthworm coelomic fluid (ECF), and artificial lysosomal fluid (ALF) to investigate the changes of cAgNPs in biological fluid contains of electrolytes. As a result, deionized water (D.W.) was more homogeneous distribution than ECF and ALF. On the other hands, cAgNPs dispersed in ECF shows high ionization and aggregation. This result means that small amounts of cAgNPs can be permeated to earthworms and can be ionized easily, so cAgNPs may cause low toxicity to earthworms exposed cAgNPs.

**580 A New Indicator to Evaluate the Toxicity of Contaminants Against Soil Microbial Community** V. Shah, Dowling College; V. Walker, N. Kumar, Queens Univ, Dept of Biology. In the field of ecotoxicology, it is hard to compare the toxicity of contaminants to soil microbial community based on the data originating from multiple studies. Using copper, silver and silica oxide nanoparticles as examples, in the study to be presented we will show the use of a new microbial community toxicity indicator (MCTI) to compare the toxicity of various NPs against microbial community. MCTI incorporates the toxicity values measured using culture dependent (total substrate utilization pattern) and culture independent (PCR-DGGE and FAME analysis). The toxicity of nanoparticles was measured against Arctic soil microbial community.

**581 Hazard Assessment and Product Recognition Drives Green**

**Chemical Design** E.T. Lavoie, US Environmental Protection Agency, Design for the Environment Program, Environmental Protection Agency, Office of Pollution Prevention and Toxics; E. Sommer, US Environmental Protection Agency. Green chemical design can be driven by comparative hazard assessment of chemicals in end use products, enabling identification of safer chemical alternatives. Recognition programs, ecolabels, criteria and standards for greener chemistry, are encouraging 'benign by design' considerations in product development. USEPA's Design for Environment (DfE) program has encouraged "safer chemistry" since 1992 by allowing use of the DfE label on products containing the safest chemicals available for a given functional use (e.g., surfactancy). In the last 6 years the program has used its experience in identifying safer chemicals to generate criteria for safer surfactants, solvents, chelants, fragrance chemicals and cleaning product chemicals in general. The DfE criteria for surfactants, as an example, is based on aquatic toxicity and biodegradation rate and has influenced manufacture of new surfactants by companies such as Dow and BASF where both functionality and toxicity were design considerations. The understanding of existing surfactants on the market, their functionality, cost, chemistry, toxicity, and persistence influenced recognition of the safer surfactants on the market, identified the key characteristics of concern, defined the bar for a 'safer' surfactant and resulted in newly designed surfactants coming to market that encompassed green chemistry principles. DfE reviews ecotoxicity and fate data in addition to human health endpoints before recognizing safer chemicals. SAR based predictive methods and safer chemical design principles are used to fill data gaps, interpret data, and determine which chemistries are not inherently safer, or how chemicals could be designed to degrade. However, encompassing both reduced toxicity and reduced persistence can have contradictory chemical design principles. This presentation will use chemical examples to illustrate criteria development, green design success and ongoing challenges for green industrial chemistry design, based on consideration of multiple health and environmental endpoints.

**582 Computational Tools to Guide Design for Degradation and the Development of Preferred Chemicals and Products**

R. Williams, Environmental Science & Green Chemistry Consulting, LLC; T. Williams, Wesleyan Univ, Chemistry. Emerging market forces highlight the importance of innovating environmentally preferred products and manufacturing methods. Innovations in organic synthesis manufacturing processes have achieved substantial environmental and economic benefits through scientific advances in tools such as greener reactions. Computational tools can inform future innovations in molecular design and formulations that result in environmentally preferred consumer products and specialty chemicals such as pharmaceuticals. Product design scientists apply their knowledge of chemical properties to construct molecular architecture and product formulations with commercially valuable functionality. Green Chemistry Principle # 10 (Design for Degradation, DfD) recommends designing molecular architecture that is both environmentally preferred and functional. Consequently, DfD has been emphasized as a long-term pollution prevention opportunity for, as an example, emerging contaminants such as pharmaceuticals. The nature of chemical product discovery and development establishes the essential role computational tools must play, the nature of the tools and paradigm to be achieved, and the scientific interactions that would be supportive if environmentally preferred chemical products are to play a more prominent role in achieving environmental quality.

**583 Identification of New and Existing PBTs by QSPR: A Greener**

**Screening** E. Papa, Univ of Insubria, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology, Univ of Insubria, DBSF; P. Gramatica, Univ of Insubria, QSAR Res. Unit Environ. Chem. Ecotox./Dep. Structural & Functional Biology. The screening of Persistent, Bioaccumulative and Toxic (PBT) chemicals is still a challenging process for scientists and regulators. The European REACH regulation requires the PBT/vPvB assessment of a substance to be carried out on the basis of the criteria defined in its Annex XIII, and promotes the use of alternative, predictive, *in silico* methods, such as Quantitative Structure-Activity (Property) Relationships (QSA(P)Rs), in order to minimize the expected high costs of experimental tests necessary for the hazard/risk assessment of chemicals, and to maximize the use of existing experimental data. Furthermore, REACH encourages the substitution of unsafe substances, such as PBTs, with safer alternatives, which is in agreement with the Green Chemistry Principles 2 and 10 (2: design safer chemicals and products, 10: design chemicals and products to

degrade after use). Molecular modeling approaches such as QSA(P)R can be successfully applied in "safe Chemical Design", in perfect line with the "benign by design" concept. In this study we describe the development and the possible use of the PBT-Index QSPR model. This robust and externally validated model is based on a multivariate screening approach which ranks chemicals according to their P, B and T properties, and is able to identify PBT-like chemicals only on the basis of their chemical structure. To further evaluate the quality of our approach, the accuracy of our results is compared with other PBT screening approaches (US-EPA PBT Profiler, the Canadian DSL List, and HAF values). A screening by consensus is also proposed, which takes into account the different assumptions characterizing each method considered for the comparison (i.e., chemical structure, partitioning and cut off criteria), and gives a more realistic assessment of the potential PBT behaviour of chemicals from different perspectives. Finally, case studies showing the possible "green" application of the proposed QSPR model to screen PBTs, their alternatives or large lists of structures are also presented. The PBT-Index QSPR model is proposed as a hazard screening tool, applicable also by regulators, for the early identification and prioritization of not yet known PBTs. This "green", structurally-based, model is applicable to identify alternatives to PBTs among the existing chemicals, or to screen libraries of unsynthesised candidates through a "safe by design" (drug design-like) approach.

**584 Chemists Provide Solutions, Green Chemists Solve Problems**

N. Gathergood, Dublin City University, School of Chemical Sciences; R. Gore, D. Coleman, M. Ghavre, M. Gurbisz, T. Hayes, I. Beadham, Dublin City University; L. Myles, S. Connon, University of Dublin, Trinity College; B. Quilty, Dublin City University; M. Spulak, Charles University; T. Garcia, IIQAB-CSIC. Last year Paul Anastas published a review highlighting recent applications of the 'Twelve Principles of Green Chemistry' proposed in the seminal book 'Green Chemistry: Theory and Practice'. In these publications both academia and industry are shown a new approach to environmental protection. The core concept presented was that it is better to 'reduce than treat' waste. One of the reasons that the chemical industry has great interest in Green Chemistry is the financial benefits. By developing greener and cleaner methods and processes, that inherently generate less waste, the costs associated with waste disposal can be reduced. Industry can reduce their environmental impact as well as increase profitability. In their book, Anastas and Warner proposed the 'Twelve Principles of Green Chemistry' primarily to inspire chemists to create environmentally less damaging synthetic methodologies. Our own research team in Dublin encompasses a variety of these green chemistry principles to realise environmentally friendly organic synthesis for industry. Concurrent assessment of the performance of a new chemical or class of reaction with its potential environmental impact enables our group to focus investigations on 'safer' and green reaction methodologies. The development of reactions with high atom economy, to reduce waste products, combined with low toxicity and biodegradable reaction media, is one example of our benign-by-design philosophy. Selected recent examples from our investigations in the fields of ionic liquids, organocatalysis and drug discovery will be presented.

**585 Assessment of Health Impacts in Munitions Research, Development, Testing and Evaluation: A Phased Approach**

M.S. Johnson, US Army Institute of Public Health, Health Effects Research Program; W.S. Eck, US Army Institute of Public Health, Health Effects Research Program. Sustaining range operations while maintaining force health is vital to our National security. Current research is underway focused in discovering munition constituents that have a negligible environmental health impact. Determining the potential for adverse environmental health effects requires an interdisciplinary approach that must be phased coincident with munition design. They must be applied in a logical, tiered way consistent with the research, development, and engineering level of effort. This process will enable researchers to determine and refine environmental health properties throughout development--through modeling and simulation in the early stages and testing in the later stages. Researchers will use this knowledge to identify and mitigate potential environmental health risks at each stage of development. Details of the process are provided and examples presented.

**586 Targeted Design of a New Effective and Biodegradable Active Pharmaceutical Ingredient**

K. Kümmerer, Leuphana Univ Lüneburg, Institut für Nachhaltige und Umweltchemie; G. Marano, Calvatis GmbH; E. Frei, M. Wiessler, Deutsches Krebsforschungszentrum. The products of

the chemical and pharmaceutical industries are ubiquitous in everyday life. For a long time, production of chemicals and pharmaceuticals was associated with heavy pollution of the environment and serious health effects. During the second half of the last century, such impact was reduced tremendously – at least in developed countries by proper measures such as effluent treatment and containment. In the meantime it has been learned that the products themselves i.e., the desired molecules and not the unwanted ones originating from side reactions are present in the environment because of intended use and pose a risk on humans and the environment. In the past pharmaceuticals and chemicals that have been used were optimized for stability. This is the reason why they are present in the environment. For a sustainable use of chemicals and pharmaceuticals, the aim is to have molecules intentionally designed for efficacy and efficiency as well as fast and complete mineralization in the environment. In a combination of experimental work and in silico tools we have optimized a new pharmaceutical lead structure in terms of efficiency of the pharmaceutical active ingredient and improved biodegradability in the aquatic environment after it reaches the aquatic environment in case of incomplete metabolism in patients. The example demonstrates that even pharmaceuticals can be designed that they are active and biodegradable and that benign by design is a promising and important building block of green and sustainable pharmacy (1). The approach and the results of this study will be presented. (1) Kümmerer K., Hempel M. (Eds.) (2010) Green and Sustainable Pharmacy. Springer, Heidelberg Dordrecht London New York

**587 Green Notes for Musks** R. Boethling, US Environmental Protection Agency, Office of Pollution Prevention and Toxics, USEPA, Office of Pollution Prevention and Toxics, MC 7406 The design of the molecule itself is the earliest phase in the process of developing useful products. Two of the 12 green chemistry principles, that chemicals should be designed to have minimal toxicity and degrade environmentally to innocuous products, are central to chemical design. Previously we used a series of case studies to show that generalizations about the effects of molecular structure can be used to design small molecules for biodegradability. Here, we extend one of the case studies (synthetic musk fragrances) to a wider range of musks, and address bioaccumulation potential and ecotoxicity in addition to persistence. To this end, we first summarize ready biodegradation data for musks including unpublished data from US Premanufacture Notice (PMN) chemicals. Bioaccumulation potential and aquatic toxicity are then estimated for this set of musks using EPA's BCFBAF, KOAWIN and ECOSAR programs (components of EPI Suite), and the results are used to compare musks across the various structural classes and make inferences about their environmental attributes. Last, the analysis is extended to untested musks including target molecules. In this way, we show how predictive methods and knowledge can be included as additional facets in rational design of small molecules.

**588 Does the Colour of Your Chemistry Matter? What Green Chemistry Can Learn from Ecotoxicology** M. Hanson, Univ of Manitoba, Dept of Environment and Geography, Univ of Manitoba, Faculty of Environment; K. Solomon, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Centre for Toxicology, School of Environmental Sciences. The sub-discipline of Green Chemistry has many laudable ideals and precepts, including the aim of reducing the toxicological footprint of chemical processes and products. Eventually though, this idealism runs into a wall of practicality and very necessary pragmatism, especially when examining its aims under an ecotoxicological lens. What might seem simple on paper, e.g., do not use or produce toxic materials, is devoid of real meaning when scrutinized closely, as all things have the potential to be toxic, and in different ways to different things. Context matters. What needs to be ascertained in advance is what level of exposure to a contaminant or response as a result of exposure, is deemed scientifically and socially acceptable. For example, pesticides are by their very nature meant to be highly toxic to selected organisms. By defining *a priori* what is and is not acceptable from an ecological perspective, you can develop and market these vital chemical tools with a reduced ecological impact. While Green Chemistry can inform ecotoxicology and risk assessment, more importantly, it needs to better understand these sciences so that it can advocate for more sustainable approaches within chemistry as a whole.

**589 The Boston Harbor Project: Documenting an Environmental Success Story** A. Rex, Massachusetts Water Resources Authority, Environmental Quality. The Boston Harbor Project began in 1985 with the creation

of the Massachusetts Water Resources Authority (MWRA). Spurred by a Federal Court Order, the outcome of a lawsuit against the state for violations of the Clean Water Act, MWRA's task was to design and build modern wastewater treatment facilities serving the 2.5 million people of greater Boston according to a stringent timetable. Over the course of the next 15 years, the practice of discharging sewage sludge to the harbor would end (1991) undersized primary treatment plants would be replaced by a modern secondary treatment plant (1998), and a new, 9.5-mile-long outfall-diffuser system would be bored through bedrock to dilute the treated effluent in the deep waters of Massachusetts Bay (2000). While the new treatment facilities were being built, another major project was initiated to control combined sewer overflows, which were discharging more than 3 billion gallons of raw waste mixed with storm water every year to the shorelines of the harbor and its tributary rivers. To measure the impacts of these multibillion-dollar projects, MWRA began its harbor and outfall monitoring programs in 1989. The harbor data would tell the story of the recovery of an ecosystem, and data collected throughout Massachusetts Bay would provide "before" and "after" comparisons to determine whether the long outfall might adversely affect the ocean around it. Effluent data show that treatment reduced the loadings of many contaminants discharged by more than 80%. Water column bacterial monitoring data show the effect of improved treatment and CSO control. The process of eutrophication appears to be reversing. The benthic community has shown dramatic changes in some parts of the harbor. A great deal has been learned about the process of recovery, and some parts of the harbor ecosystem have recovered more quickly than thought.

**590 Twenty Years of MWRA Water Quality Monitoring – Harbor vs. Bay Outfall Observed Trends in Nuisance Species** P.S. Libby, Battelle, Environmental Solutions; D.G. Borkman, Univ of Rhode Island, Graduate School of Oceanography; D.M. Anderson, Woods Hole Oceanographic Institution, Dept of Biology. On September 6, 2000, the Massachusetts Water Resource Authority (MWRA) transferred their effluent discharge from Boston Harbor to an outfall in Massachusetts Bay. Limited ecological impact was predicted for the transfer and has been validated by the long-term MWRA monitoring program (1992–2011). After ten years of post-diversion monitoring, changes in the nutrient regimes in the harbor and bay are unambiguous – dissolved inorganic nitrogen (DIN) levels have decreased in the harbor and coastal waters while increasing locally near the bay outfall. In Boston Harbor, the dramatic decrease in DIN has been associated with significant decreases in chlorophyll and particulate organic carbon, lower primary production, and changes in seasonal primary productivity patterns. In the bay outfall area, there is no indication that the higher nutrient loads have translated into significant changes in phytoplankton biomass, whether measured as chlorophyll, particulate organic carbon, or abundance. Contingency Plan thresholds were established to evaluate potential changes in the magnitude of nuisance phytoplankton species blooms. These include *Alexandrium fundyense* a paralytic shellfish poison producing species, *Pseudo-nitzschia* spp. some of which produce domoic acid, and *Phaeocystis pouchetii*, which is considered a nuisance species due to its potential for very large blooms. Blooms of all three species are observed in Massachusetts Bay and during post-diversion monitoring there have been apparent changes in the frequency, magnitude, and duration of these blooms in the Bay. The major 2005 red tide (*A. fundyense*) renewed interest in the potential effect the outfall may have in exacerbating such blooms, however, associations between the bay outfall and regional phytoplankton blooms have not been observed. This presentation briefly summarizes the overall water quality findings of the MWRA monitoring program, details the trends observed for these three nuisance species, and assess the relative impact of the MWRA outfall on these blooms.

**591 Testing for Discharge-related Changes in Massachusetts Bay In-faunal Communities** K. Keay, Massachusetts Water Resource Authority; J. Blake, N. Maciolek, AECOM; W. Smith, Temple Univ; D. Dahlen, Battelle. The Massachusetts Water Resources Authority studies benthic communities in Massachusetts Bay as part of an ambient monitoring program required under the National Pollution Discharge Elimination System (NPDES) discharge permit for its offshore effluent outfall, which came online in September 2000. Soft-sediment sampling for contaminants, infauna, and tracers at 23 "nearfield" stations within 7 km of the discharge and 8 reference stations spread from Cape Ann to Cape Cod has been conducted annually since 1992, providing 9 years of baseline sampling and 10 years of discharge monitoring results through August 2010. Testing for the effects of secondary



effluent discharge on sediments and benthic communities in this dispersive and dynamic coastal environment has been challenging. No discharge-associated changes in community composition, abundance, or diversity have been observed, nor have contaminant concentrations increased. Sensitive BACI analyses on contaminant, tracer and infaunal data have confirmed that only levels of the spore-forming bacterium *Clostridium perfringens* in sediments show strong evidence of an increase related to outfall discharge. MWRA's NPDES permit requires that, as soon as they pass QA/QC checks, raw monitoring data be tested against metrics designed to detect changes from baseline conditions substantial enough to warrant rapid evaluation and notification of regulators. In January 2011, for the first time, infaunal data from MWRA's benthic monitoring exceeded these "thresholds". Two of the four biodiversity parameters monitored, Shannon-Wiener  $H'$  and Pielou's  $J'$ , were slightly higher than the threshold in nearfield sediments. Our ongoing evaluation of these exceedances will be considered and presented. Evaluations conducted as of May 2011 suggest that the 2010 infaunal results reflect natural variation in infaunal community composition and diversity, rather than a discharge-related change in the communities.

**592 Boston Harbor Cleanup Brings Back the Benthos** R. Diaz, Virginia Institute of Marine Science. Not long ago, nutrient loadings to Boston Harbor were among the highest in the world. A major overhaul of sewage treatment in the area, including treatment upgrades and relocation of an outfall offshore in 2000, was designed to address this problem and clean up the harbor. The cleanup plan resulted in a reduction of organic matter load to the harbor of about 90%. The reduction in carbon loading associated with the outfall relocation led to a shift to more aerobic conditions in the harbor. This shift paved the way for an increase in *Ampelisca* amphipods, which need some level of organic enrichment but better water quality than the "old" Boston Harbor could offer. The cover of amphipod mats increased from < 20% in 1992 to >60% in 1995-1998. As conditions continued to improve, carbon loading fell below the optimum needed to sustain the amphipod mats (the "tipping point" appears to be about 500 g C per m<sup>2</sup> per y), and they slowly disappeared, reaching < 20% coverage by 2000 and disappearing by 2005. While they dominated the benthos, amphipod tubes facilitated an increase in biodiversity and bioturbation, which increased the depth of oxidized sediments, improving conditions for other benthic invertebrates. These results highlight the harbor's good news: wastewater treatment improvements have tipped the balance back to good benthic habitats by favoring organisms that enhance bioturbation. However, some areas of the inner harbor, especially near combined sewer outfalls (CSO), have not yet experienced much improvement.

**593 Decreases in the Prevalence of Liver Disease in Boston Harbor Winter Flounder** M. Hall, Massachusetts Water Resources Authority, Environmental Quality Dept; M. Moore, Woods Hole Oceanographic Institution. The high prevalence of liver neoplasm (tumor) in winter flounder (*Pseudopleuronectes americanus*) collected from Boston Harbor in 1984 was one of several important findings which resulted in the Boston Harbor Project. Since 1991 flounder have been collected from several locations in the Boston Harbor, Massachusetts Bay, and Cape Cod Bay regions during April or early May. The livers of 50 fish from each site are analyzed for various histological markers of disease. Edible meat tissue and liver tissue from 15 fish (3 replicates of 5 fish each) are analyzed for chemical contaminants – mainly organic contaminants in edible meat and organic and inorganic contaminants in livers. Results show a steady decrease in tissue concentrations of organic contaminants at all sites. Metals measured in liver tissue have been highly variable, spatially and temporally, and show no conclusive trends. Liver tumors have not been observed in fish collected from Boston Harbor since 1996. The prevalence of Centrotubular Hydropic Vacuolation (CHV), a precursor to liver tumors, has declined throughout the harbor and western Massachusetts Bay region. The prevalence in harbor flounder is now half of what it was during the early 1990s.

**594 A Before-After-Control-Impact Study of Changes in Contaminants in Winter Flounder, Lobster, and Caged Mussels in Massachusetts and Boston Harbor** S. Kane Driscoll, M. Edwards, Exponent Inc.; E. Nessler, A. Pembroke, Normandeau Associates. This study addresses various questions related to the Fish and Shellfish component of the Massachusetts Water Resources Authority's (MWRA) Harbor and Outfall Monitoring program for Boston Harbor and Massachusetts Bay. In September 2000, MWRA moved the outfall that discharges wastewater from the MWRA

Deer Island Treatment Plant (DITP) in Boston Harbor to a new outfall diffuser system located in Massachusetts Bay 9.5 miles from the DITP. The goal of this study was to examine whether levels of contaminants in fish and shellfish have changed as a result of the relocation of the outfall, and whether current levels of contaminants in fish and shellfish are significantly different among organisms collected from the area of the outfall, Boston Harbor, and reference sites in Cape Cod Bay. A secondary goal was to examine whether current levels of contaminants in the edible tissue of fish and shellfish collected near the outfall present a risk to human health or to the environment. Two approaches were taken to evaluate whether concentrations at the outfall site were significantly different from other locations and times. A before-after-control-impact model was used to assess whether concentrations prior to the relocation of the outfall were significantly different from concentrations after the relocation, and whether the differences were related to the relocation of the outfall. Additionally, data for each year following the outfall relocation were evaluated using an analysis of variance to compare concentrations among locations. As expected, concentrations of some contaminants in caged mussels deployed in the vicinity of the new outfall increased after the effluent was diverted to the outfall in 2000, even after taking into account changes at the control site in Cape Cod Bay. Concentrations of contaminants in lobsters and flounders at the new outfall site did not increase as a result of the relocation of the outfall. Concentrations of most contaminants were below risk-based concentrations, although concentrations of PCBs exceeded a conservative EPA risk-based concentration at all locations, indicating a broader regional distribution of PCBs.

**595 The Boston Harbor Project and the Reversal of Eutrophication of Boston Harbor** D.J. Taylor, Massachusetts Water Resources Authority, Environmental Quality Dept. Boston Harbor, an urban bay-estuary in the northeast USA, has recently been the site of a large wastewater project that culminated with the diversion of its wastewater discharges offshore. The project, which was widely referred to as the Boston Harbor Project (BHP), provided an opportunity to document the effects of large changes in nutrient inputs on a highly-flushed bay-estuary, of the type common along the northeast coast of North America. Less is known of the effects of enrichment of these types of systems than of the less-flushed bays and estuaries that occur further south. Before the BHP, the total inputs of N and P to Boston Harbor were among the highest for bays or estuaries in the USA. The project decreased the harbor N and P inputs by between 80% and 90%. The large decreases in inputs triggered changes to the harbor water-column and sediments, its seagrass and benthic invertebrate communities, and to its C and N budgets. For certain components of the ecosystem the changes were rapid, and have been sustained through the full 10 years since diversion. For others, the changes have been much slower, and are still underway.

**596 Trace Metals in Boston Harbor; a Little More than Two Decades of Observations** C. Krahforst, G. Wallace, University of Massachusetts Boston, Environmental Earth Ocean Sciences. In the mid-1980's, the City of Boston was federally-mandated to implement measures that would return Boston Harbor to standards specified by the US Clean Water Act. The clean-up project began by mitigating direct discharges of municipal wastewater to the Harbor. In 1987, sludge disposal to the Harbor ceased. In 2000, the last phase of necessary infrastructure was completed and 61 metro-Boston communities (2.5 million people) began discharging wastewater at a site in Massachusetts Bay located 16 km east of Boston Harbor. At this point, the total cost of the clean-up was \$3.9 billion. Noticeable water quality improvements in Boston Harbor were observed shortly after and have been linked to this clean-up effort. However, routine monitoring of some contaminants accumulated by resident *Mytilus edulis* (blue mussel) show Boston Harbor to be well elevated for Pb, Cu, Zn, polyaromatic hydrocarbons, polychlorinated biphenyls, dieldrin, and other classes of compounds when compared to other monitoring sites along the Gulf of Maine (see <http://www.gulfofmaine.org/gulfwatch/>). Water samples collected during five surveys of 24 stations in Boston Harbor between 1986 – 2002 show most dramatic changes in metal water quality after the sludge discharges were discontinued in 1987. However since that period, during which metro-Boston wastewater underwent improved treatment and direct discharges to Boston Harbor ceased, trace metal concentrations in filtered Boston Harbor water show little or no improvements. While trace metal (Pb, Cu, Zn, Fe) concentrations exhibited predictable spatial variability (water samples from the inner harbor were distinctly higher than outer harbor samples), mean metal concentrations in Harbor water remained remarkably unchanged (Inner Harbor Pb and Cu

concentrations:  $0.6 \pm 0.12$  and  $23 \pm 3$  nmol kg<sup>-1</sup>, respectively). The results from these surveys will be compared to a recent 2011 Boston Harbor survey to aid assessments of the changing trace metal sources as the Harbor recovers from decades of direct wastewater discharges. These results may also be useful in future ecosystem management considerations. For some contaminants, flux from contaminated sediments in Boston Harbor may be comparable to their loadings from wastewater discharges to the Harbor during the 1990s.

#### 597 Incorporation of WHAM VI Binding Reactions and Metal Sulfide Oxidation Kinetics into TICKET-UWM

**K.J. Farley**, Manhattan College, Civil and Environmental Engineering, Manhattan College, Civil & Environmental Engineering; **P.M. McMahon**, Manhattan College, Civil and Environmental Engineering; **K.J. Rader**, Mutch Associates; **R.F. Carbonaro**, Manhattan College, Civil and Environmental Engineering, Manhattan College, Dept of Civil and Environmental Engineering. The tableau input coupled kinetic equilibrium transport-unit world model (TICKET-UWM) has been developed as a screening model for assessing the combined effects of transport, chemical speciation and kinetics on metal releases to lakes. Chemical speciation reactions in the original model were assumed to follow the Windermere Humic Acid Model Version V (WHAM V). This decision was made in large part based on the use of WHAM V in the Biotic Ligand Model (BLM). In addition, metal sulfides in the model were assumed to oxidize instantaneously upon resuspension in the water column. These two assumptions are currently being re-evaluated as follows. First, a revised parameterization of WHAM, WHAM VI, was developed to provide a better prediction of the competitive reactions of protons and metals with natural organic matter (NOM). The WHAM VI parameterization was therefore incorporated into TICKET-UWM for testing. As part of this evaluation, the WHAM VI, TICKET-UWM results are being compared to metal partitioning data and metal removal rates for Lake Coeur d'Alene, Idaho to serve as a preliminary validation of the model. Second, a review of metal sulfide oxidation rates was performed to provide an overview of oxidation rates for various metal sulfides. A second-order kinetic rate law was added to TICKET-UWM to simulate metal sulfide oxidation in the water column and a comparative evaluation is being performed to determine the overall effect of oxidation kinetics on the cycling and bioavailability of metals in lakes.

#### 598 The Natural and Industrial Cycling of Indium in the Environment

**S.O. White**, H.F. Hemond, MIT, Dept of Civil & Environmental Engineering. Indium is a metal whose production is increasing dramatically due to new uses in the rapidly growing electronics, photovoltaic, and LED industries. Little is known, however, about the natural or industrial cycling of indium or its environmental behavior or toxicology. Such information is needed in order to anticipate and mitigate potential adverse environmental impacts. The combination of materials flow analysis and environmental geochemistry collectively comprise what has been called the "anthrobiogeochemical cycle". When studies of the flow of an element through industry and the environment suggest that its anthropogenic fluxes are becoming comparable to its natural fluxes, Klee and Graedel (2004) have suggested that the element should be flagged for priority study as having potentially harmful effects. This concept is particularly useful for assessing the impact of metals newly introduced to mass industrial use, for many of which the toxicological and ecotoxicological effects have not yet been established. Our studies of the dominant natural and industrial fluxes of indium show that industrial emissions of indium to the environment are already larger than natural emissions, and suggest that metal smelting and coal burning are its primary industrial sources. Because atmospheric releases are thought to be large but are poorly quantified, and because inhalation is thought to be an important exposure pathway for indium, we have studied indium in airborne particulate matter in the Northeastern United States. These studies have shown that indium concentrations in 5 locations from Boston, MA to Rochester, NY vary from below detection limits to 6 pg/m<sup>3</sup>. There are significant differences between samples from different locations and over the course of a year. Atmospheric back trajectories generated with NOAA's Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLOT) model suggest that the highest indium concentrations come from the north, potentially from smelting operations, while lower concentrations are seen in air traveling from the midwestern US. Further work to correlate indium concentrations with other metals and to obtain smelting emissions data will help to further elucidate the sources of indium to this region.

#### 599 Comparison of Models for Metal Partitioning to Natural Organic Matter: Chemically-based Model and WHAM VI

**Y.B. Atalay**, Cardno Entrix, Civil and Environmental Engineering Dept; **R.F. Carbonaro**, Manhattan College, Civil and Environmental Engineering, Manhattan College, Dept of Civil and Environmental Engineering; **D.M. Di Toro**, Univ of Delaware, Dept of Civil Engineering. The WHAM VI model of metal-DOC partitioning was compared to the Excel version of WHAM VI, coded in visual basic with equilibrium constants derived from Irving-Rossotti Linear Free Energy Relationships, IR-LFERs. The Excel version was altered to use Irving-Rossotti slopes,  $\alpha_O$ , and  $\chi_{OO}$ 's for oxygen functional groups to compute all the metal-NOM (Natural Organic Matter) binding constants. The WHAM VI framework including IR-LFERs was called WQL-IR, shorthand for WHAM-EQL-IR, after the MINEQL series of programs. The main issues addressed are the relationships between the WHAM VI and IR-LFER metal-DOC binding constants, and testing WQL-IR by comparing model predictions with WHAM VI, and with field data sets. The model-model comparisons are performed for humic and fulvic acids at widely varying metal ion activities for pH values of 5, 7 and 9 for Ca<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup> metal ions. Data sets of field measurements for Ca<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup> were compiled from the literature together with metal vs. metal competition experiments. Comparisons of the WQL-IR models and WHAM VI are presented using model-model and model-field data comparisons.

#### 600 From Exhaust Pipe to Plant: Tracking the Fate of Trace Metal Emissions in Roadside Gardens in Toronto, Canada

**C. Wiseman**, Univ of Toronto, Centre for Environment. Much attention has been paid to the development of more reliable soil quality guidelines which consider the various factors that influence metal speciation and bioavailability. A great deal of what we currently know about soil metal dynamics and speciation has been derived from controlled lab studies, using artificially amended soils. However, some evidence suggests that metals present in field soils, which have undergone natural aging processes, may be less bioavailable. Another issue which has been largely ignored to date is the potential for the direct uptake of metals through atmospheric deposition, in addition to the better known soil-plant uptake route. This research explores these issues as part of a long term study which focuses on the fate of traffic-related trace metal emissions and their uptake by plants grown in Toronto, Canada. Oregano (*O. heracleoticum*), eggplant (*S. melongena*) and beets (*B. vulgaris*) were cultivated at several locations with predicted variable metal inputs beginning in 2010. Following a microwave-assisted acid digestion procedure, samples are measured using ICP-MS. Preliminary results from the first phase of this study to examine trace element stabilization processes in aging field soils suggest several important trends. First, there is a high degree of variability in traffic-related metal accumulation over time at the site where the soil was remediated. Decreases in certain metal concentrations such as Cu, after an initial accumulation, appeared to be related to such factors as organic matter loss following remediation. Second, there was a high degree of variability in metal uptake by plants as a function of soil age. For instance, Cd was more readily taken up by oregano plants grown in freshly remediated soils compared plants grown in older aged soils at a heavy traffic location. Third, elevated concentrations of certain elements measured in plant tissues compared to soils such as Cd suggest metal uptake through atmospheric deposition in addition to soil-plant transfers. The next phases of this research will continue to explore current trends, as well as examine the role of mineralogical controls in governing the fate of metals from the solid to liquid phase.

#### 601 Geochemistry of Chromium and Nickel in Sediment of California Embayments Suggest Natural (Ultramafic) Origins

**S. Clough**, Haley & Aldrich, Inc, Risk Assessment, Haley & Aldrich, Inc; **J. Parkin-Kullman**, Haley & Aldrich, Inc., Risk Assessment. Investigations of facilities for potential impacts to sediment often call for a statistical evaluation of "Site" concentrations relative to "Background" conditions. These comparisons often result in false positives because of a lack of statistical power, which can be due either to an inadequate background dataset or a right-skewed bias in the data for the Site. Simple graphical presentation of geochemical relationships between naturally occurring metals, which allow for the visualization of potential correlations, can prove a better (and easier to understand) approach than more traditional statistical evaluation methods. The magnitude of the importance of this assessment is related to the selection of Constituents of Concern (COCs) for a Site, which typically hinges on whether concentrations found in Site sediment exceed identified

Background levels. Recent sediment chemical data obtained from a San Francisco industrial facility underlain by a serpentine (ultramafic) bedrock formation revealed that concentrations of both nickel and chromium were greatly elevated. Serpentine bedrock, however, is widely known to be naturally enriched in both of these metals, indicating it as the potential source that would be considered a Background condition. Because of the ultramafic nature of the California (CA) coastline in general, the CA Sediment Quality Objectives database was further mined to examine the potential to establish relationships between chromium, nickel, and other matrix metals. Graphs of concentrations of chromium and nickel in sediment samples from all of the major CA embayments revealed a surprisingly consistent correlation between these two metals based on the available data. Furthermore, plots of the ratios of chromium and nickel concentrations for the embayments normalized using either aluminum or iron concentrations for the associated samples, showed a remarkably "vertical" character, validating the consistency of this relationship. These observations will be compared to similar analyses conducted for datasets of sediment chemical data from East Coast harbors. The efficacy of using nickel and/or chromium concentrations as benchmarks for discerning Site-related metals contamination versus background in West Coast sediments will also be discussed.

**602 Linear Free Energy Relationships for Describing Metal Binding to Nitrogen Functional Groups** R.F. Carbonaro, Manhattan College, Civil and Environmental Engineering, Manhattan College, Dept of Civil and Environmental Engineering; Y.B. Atalay, Cardno Entrix, Civil and Environmental Engineering Dept; D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering. Nitrogen functional groups are an important constituent of natural organic matter (NOM) and are known to be responsible for complexation of metal ions in aquatic and terrestrial environments. Their role in metal partitioning to NOM was investigated through the development of linear free energy relationships (LFERs) for small organic molecules containing nitrogen functional groups. LFERs for estimating the metal-ligand stability constants,  $\log K_{ML}$ , were developed using a data set of 57 monodentate and 101 bidentate ligands for 6 metal ions:  $Mn^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$  and  $Cd^{2+}$ . Monodentate and bidentate  $\log K_{ML}$  values were adequately described using the Irving-Rossotti LFERs previously developed for ligands containing negatively-charged oxygen functional groups. Corrections to the LFER equations were necessary to account for steric hindrances to metal complexation affected by primary, secondary, tertiary amines. Predictive capabilities of the LFERs were excellent as RMSE values for each metal were less than 0.5 log units. The resulting LFER equations can be used to estimate  $\log K_{ML}$  values for monodentate and bidentate ligands containing neutral nitrogen donor groups where such values do not currently exist in the literature. Comparison of these results to our previous work with negatively-charged oxygen donor atoms reveals that nitrogen functional groups are comparable in strength to their oxygen counterparts for most metal ions.

**603 Method Comparisons for Copper Speciation in Salt Water and Implications for Bioavailability Model Development** S. Smith, Wilfrid Laurier Univ, Dept of Chemistry, Wilfrid Laurier Univ, Chemistry; R. Diamond, T. Tait, Wilfrid Laurier Univ, Chemistry; R. Santore, HDR|HydroQual; C. Wood, McMaster Univ, Dept of Biology. Determination of copper speciation is essential to understand and predict copper bioavailability in saltwater environments. In particular, copper speciation methods must be representative of true speciation at copper levels toxic to organisms in marine and estuarine systems. In the literature, several speciation techniques are proposed but there is a need for side-by-side method comparisons utilizing the same set of samples. Towards this end, four different marine samples were collected from a variety of locations and copper speciation was determined using four distinct methods: direct anodic stripping voltammetry (ASV), competitive ligand voltammetry, ion selective electrode (ISE) and fluorescence quenching. For ISE it was found necessary to utilize flow through apparatus to have sufficiently low detection for quantification of ambient free copper. In general, direct voltammetry overestimates the amount of free and inorganically complexed copper compared to ISE. For example, using ISE a Florida bayou sample was found to have free copper of  $10^{-10.3}$  whereas ASV determined a labile copper concentration of  $10^{-8}$  mol/L. Direct voltammetry detects any copper species labile enough to react at the mercury electrode ( $Cu^{2+}$ ,  $CuOH^{+}$ ,  $CuCl^{+}$ , ...) whereas the ISE only responds to free cupric ion. If the ISE free copper is assumed to be correct, chemical equilibrium calculation, with chloride, hydroxide and

carbonate complexation, predict ASV-labile copper of  $10^{-9.1}$  mol/L, which is still an order of magnitude lower than the measured value. Likely, weak organic complexes are also being detected by the ASV method. Fluorescence quenching titrations on these same samples predict copper speciation consistent with ISE analysis. Accurate and reliable Cu speciation estimates are critical for input to bioavailability models and the reliability of electrochemical methods need to be established by multi-technique measurements on the same samples. Funded by NSERC, ICA and CDA to CMW and DSS

**604 The Mobility, Bioavailability, and Human Bioaccessibility of Trace Metals in Urban Soils** X. Luo, The Hong Kong Polytechnic Univ, Dept of Civil and Structural Engineering; S. Yu, Institute of Urban Environment, Chinese Academy of Sciences; X. Li, The Hong Kong Polytechnic Univ, Dept of Civil and Structural Engineering. Mobility, effective and potential bioavailability, and human bioaccessibility were combined in evaluating the integrated ecological and health risks of heavy metals (Cu, Pb, Zn) in contaminated urban soils of Hong Kong. Different metals and even the same metal showed different soil-specific patterns for various environmental availabilities. Anthropogenic Pb in urban soils tended to be environmentally available. The various available concentrations (mg kg<sup>-1</sup>) and availabilities (%) of heavy metals in urban soils could be estimated from their relationships with the total metal contents and major soil parameters, such as pH, organic matter, EC, and soil texture. To make use of the total concentrations of heavy metals in urban soils, multiple regression equations predicting various availabilities of metals to plants, organisms, and humans, as well as the leaching potential to groundwater were developed from the studied soils. Taking into account the role of land use in the exposure of soil pollutants to humans, the introduction of site-specific available concentrations might give more realistic estimations of the risks posed by metal contamination to human health and ecosystem. The results would facilitate producing more applicable guidelines for risk assessments and soil remediation.

**605 Sources of PAH in Arctic Offshore Oil and Gas Development Areas** J. Neff, Neff & Associates LLC; J.S. Brown, Exponent Inc. There has been extensive exploration for and some development of oil and gas resources in Arctic waters of Alaska, Canada, Norway, and Russia. Monitoring in these development areas in the last 10 years has shown that polycyclic aromatic hydrocarbons (PAH) are abundant in Arctic river and offshore sediments, coastal peat, and the tissues of marine animals. Total PAH concentrations in continental shelf sediments of the Chukchi and Alaskan and Canadian Beaufort Seas range from about 10 to 4,500 ng/g dry wt (parts per billion). Highest concentrations are off major rivers (e.g., the Macenzie and Colville Rivers) and at a few exploratory well sites. Concentrations in Barents Sea sediments range from about 20 to 1,500 ng/g. Fingerprinting indicates that the sediment PAH are from a mixture of petrogenic, pyrogenic, and biogenic sources. The PAH in surface sediments at former exploratory well sites are enriched in perylene, indicating the PAH are primarily from peat and kerogens from rivers draining the extensive peat bogs along the Arctic Ocean. Total PAH concentrations in bivalve mollusks and benthic crustaceans from the study areas range from 20 to 360 ng/g, suggesting that sediment PAH have a low bioavailability. There is evidence of PAH from diesel fuel in sediments at a few locations, probably from accidental discharges from commercial vessels involved in offshore oil exploration and development operations. Most of the petrogenic PAH in Arctic sediments and benthic invertebrates probably are primarily from the abundant natural seeps, oil shale, and coal deposits along the Arctic coasts.

**606 Trophic Transfer of PAH Metabolites in Short Aquatic Food Webs** V. Carrasco Navarro, Univ of Joensuu, Faculty of Biosciences, Univ of Eastern Finland, Dept of Biology; M.T. Leppanen, Univ of Eastern Finland, Dept of Biology; J. Honkanen, Akvaplan-niva, Environment & Petroleum Research Group; J.V. Kukkonen, Univ of Eastern Finland, Joensuu Campus, Dept of Biology. Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants in the aquatic environment. Opposite to other Persistent Organic Pollutants (POPs), the current understanding is that PAHs are not prone to biomagnification along the trophic chain. The low potency for biomagnification is suggested to be a result of the higher biotransformation and excretion capabilities at higher trophic levels. Although the biotransformation of PAHs may lead to the formation of metabolites that could be more toxic than the parent compound, there is not abundant information about the trophic transfer of biotransformation products. Using pyrene as a model PAH, the trophic transfer of its biotransformation products was



tested in aquatic laboratory exposures. *Lumbriculus variegatus* (Oligochaeta) and *Chironomus riparius* (Diptera: Chironomidae) were exposed to pyrene in water and used as prey fed to the predators brown trout (*Salmo trutta*) and the arctic seawater amphipod *Gammarus setosus*. By using high performance liquid chromatography (HPLC) it was determined that one phase II biotransformation product produced by *L. variegatus* is transferred to *S. trutta* through diet. Similar results were found in studies conducted with *G. setosus* as predator and *C. riparius* and *L. variegatus* as prey. Additionally, pyrene and total body burdens of prey and predator analyzed through HPLC and Scintillation Counting (SC) are in accordance with the transfer of metabolites, showing that there are more mols of metabolites in predators' tissues than mols of parent pyrene ingested, indicating that necessarily a transfer of at least part of the metabolites occurred. The results highlight the fact that also the PAH metabolites should be taken into account when assessing the overall risk caused by PAHs.

#### 607 Evaluating the Toxicity of Petroleum in Arctic and Other Aquatic Environments P.M. Chapman, Golder Associates Ltd; P.M. Landrum,

Southern Illinois Univ; J. Neff, Neff & Associates LLC; D.S. Page, Bowdoin College, Dept of Chemistry. Aquatic toxicology has evolved from an initial evaluation of individual chemicals to evaluations of mixtures of chemicals and complex substances. However, experimental designs for evaluating the toxicity of complex mixtures such as petroleum in aquatic environments can be highly variable and, if not appropriate, can and have produced data that are difficult or impossible to interpret accurately. We provide guidance for the proper design of laboratory and field assessments of the toxicity of mixtures of low-solubility organic chemicals such as comprise different petroleum products, based on two fundamental requirements: establishing a dose-response relationship; and, determining the causative agent(s) of any observed toxicity. These two requirements can be met only if exposure conditions and measurement endpoints are appropriate, modifying factors are considered (e.g., test conditions, test organism life stage, chemical transformations, mixture dilution, sorbing phases), and concentration/dose response relationships are correctly interpreted. Problems that occur when these two fundamental requirements are not met are illustrated using case studies from the Arctic marine environment.

#### 608 Toxicity of Physically and Chemically Dispersed Petroleum to Arctic Species W. Gardiner, NewFields LLC; J.Q. Word, NewFields, Applied

Environmental Sciences, Newfields Northwest, Applied Environmental Sciences; K. McFarlan, R. Perkins, Univ of Alaska Fairbanks. A joint industry program was formed to investigate the toxicity of chemically and physically dispersed oil to valuable ecosystem components of the Beaufort and Chukchi Seas. Sensitive life stages of the copepod *Calanus glacialis*, polar cod (*Boreogadus saida*), and sculpin (*Myoxocephalus* sp.) were collected from the Beaufort and Chukchi Seas near Barrow Alaska and tested in laboratory facilities at the Barrow Arctic Sciences Consortium. Toxicity tests were completed using methods previously established by CROSERF, with modifications to represent conditions in ice-free, Arctic waters. Three preparations of fresh Alaska North Slope (ANS) crude oil were evaluated: physically dispersed oil, chemically dispersed oil, and physically dispersed oil under increased mixing energy conditions. Tests were conducted as spiked-tests with a half-life of four hours at 2°C to simulate environmental conditions. Total petroleum hydrocarbons (TPH), alkanes, and polyaromatic hydrocarbons PAH were analyzed by gas chromatography/mass spectrometry (GC/MS) at the beginning of each test. The mean LC<sub>50</sub>s for chemically-dispersed oil were 55 mg/L TPH (arctic cod); 22 mg/L TPH (early-season copepods); 62 mg/L TPH (late season copepods) and 27 mg/L TPH (larval sculpin). Under low mixing energy conditions, the physically dispersed ANS did not produce enough dissolved TPH to elicit a response from the copepods, but the TPH was sufficient to provide a mean LC<sub>50</sub> for the arctic cod and sculpin of 1.6 mg/L and 2.2 mg/L TPH, respectively. Under increased energy mixing conditions, the physically dispersed oil proved to be significantly more toxic to both the arctic cod and early season copepod with mean LC<sub>50</sub>s of 3.3 and 3.7 mg/L TPH, respectively. Comparison of the LC<sub>50</sub>s obtained from this study with previous studies performed on temperate and non-arctic species shows that the arctic cod, copepod, and sculpin were similarly sensitive to chemically dispersed oil than their temperate and non-arctic counterparts.

#### 609 The Arctic Copepod *Calanus glacialis* Is Less Sensitive to Acute Oil Exposure than its Boreal Sister Species *Calanus finmarchicus* B. Hansen, SINTEF Materials & Chemistry, Marine Environmental Technology; D.

Altin, BioTrix; S.F. Rorvik, I.B. Overjordet, A.J. Olsen, Dept of Biology, NTNU; T. Nordtug, SINTEF Materials and Chemistry. The use of temperate species to assess the risk of chemical toxicity to Arctic species may be difficult, because different temperatures and differences in organism lipid content affect uptake/depuration kinetics and the subsequent toxic responses. There have so far only been a few attempts to determine whether temperate effect levels are in fact applicable to polar biota. In order to address these issues we conducted a comparative study using two closely related calanoid copepod species; *Calanus finmarchicus* and *Calanus glacialis*. *C. finmarchicus* is considered an Atlantic copepod, whereas the *C. glacialis* is considered an Arctic copepod, and the experiments on *C. finmarchicus* and *C. glacialis* were conducted at relevant temperatures for the two species; 8°C and 2°C, respectively. Copepods were exposed to water-soluble fraction of fresh and weathered crude oil in three differently designed acute experiments. Firstly, LC50 levels for both contaminants were determined for both species. Secondly, experiments were conducted applying sub-lethal exposures at three different concentrations to both species followed by sampling after 12, 24 and 48 hrs. Sampled copepods were analyzed for expression of stress genes (e.g., glutathione S-transferase). Finally, we conducted experiments revealing the relationship between lipid content and acute toxicity to WSF exposure. Clear differences were shown in terms of acute toxicity, and the WSF of the fresh and weathered crude oil was more toxic to the Atlantic copepod. This may be attributed to differences in temperature optimum and lipid content of the two species. A suspected lipid content-dependent alteration of acute toxicity was verified, since for both species lipid-rich copepods survived longer than lipid-poor copepods. We also observed differences between the two species and also between lipid-poor and lipid-rich copepods in the ability to express stress genes following exposure. In conclusion, in terms of effect limits to oil exposure, the boreal copepod can be used as a model species for Arctic copepods without underestimating acute oil toxicity.

#### 610 Indigenous Microorganisms Degrade Oil in Arctic Seawater K. McFarlan, Univ of Alaska Fairbanks; M. Leigh, Univ of Alaska Fairbanks,

Biology and Wildlife; R. Perkins, Univ of Alaska Fairbanks, Civil and Environmental Engineering. A number of techniques can be used to respond to a marine oil spill such as mechanical recovery, in situ burning, the application of dispersants and natural attenuation. The success of the latter two options relies upon biodegradation by indigenous microflora. As oil exploration expands in offshore Arctic regions, it is imperative to assess the potential rate and extent of oil biodegradation in arctic marine environments by indigenous microflora under environmentally relevant conditions, including at low temperatures. A respirometry experiment validated with chemical analyses was conducted in order to determine the potential for indigenous arctic marine microorganisms to biodegrade Alaskan North Slope (ANS) crude oil in the presence or absence of a dispersant (Corexit® 9500), both in fall and late winter/early spring conditions. Sea water was collected from the Beaufort and Chukchi Seas to provide an indigenous consortium of biodegrading microorganisms. Tests were conducted at an on-site laboratory (Barrow, Alaska), at seasonal temperatures (2°C and -1°C) with minimal nutrient addition. Manometric respirometry combined with GCMS chemical analyses was used to evaluate the rate and extent of biodegradation. Biodegradation and mineralization occurred in fresh and 20% weathered ANS crude at both 2°C and -1°C with indigenous Arctic microorganisms. The presence of Corexit 9500® was not inhibitory to natural microbial degradation. The addition of Corexit 9500® enhanced the degradation of both fresh and weathered oil both in the presence and absence of low-level nutrients.

#### 611 Microbial Metagenomics as an Environmental Monitoring Tool for Offshore Oil Exploration and Production B. Metzger, Battelle. Microbial

metagenomics—the study of the genomic profile of entire microbial communities including cultured and uncultured organisms—is a powerful tool to assess widely diverse ecosystems to detect perturbations in and monitor the recovery of microbial communities. The cost of nucleic acid sequencing is rapidly decreasing, making metagenomics competitive with traditional microbial monitoring, while providing substantially more community-based and unbiased information than traditional microbial approaches. In this study, we examined the microbial metagenomic profile in three distinct environments: marine, human, and sediment. In the first case we demonstrated the application of bioinformatics data mining to identify hydrocarbon-degrading bacteria and associated functional genes in marine waters using publically available data. Examination of the Sorcerer II Expedition genetic

data revealed putative oil-degrading bacteria and related functional genes at several distinct marine locations not obviously impacted by oil-drilling operations, suggesting exposure of normal marine microflora to petroleum. The second case study focuses on the metagenomic profile of human saliva to identify microbial diversity in the normal microflora. Human saliva samples were whole-genome sequenced and after filtering out human sequences, the relative abundance of genetic sequences from prokaryotic organisms was determined using the Basic Local Alignment Sequence Tool with specified parameters. More than 20 known individual bacterial species were identified including *Streptococcus thermophilus*, a bacterium not considered part of the normal human microflora. Instead, this unique organism most likely correlated with the recent consumption of an industrially processed dairy product, illustrating the power of metagenomics to detect temporary changes in normal microflora. The third case study used novel bioinformatics processing to compare the microbial metagenomic profile in geographically identical sediments to assess impacts of Alaskan crude oil on microbial diversity and functional gene expression, resulting in a unique microbial response pattern that may be applied to future predictions or examinations of exposure to and recovery from potential hydrocarbon contamination. Further, this study sought to extend microbial environmental monitoring using transcriptomic and proteomic approaches.

**612 Attu Island – Where WWII Continues to Wage War on the Environment** D.D. Rudis, US Fish & Wildlife Service, Ecological Services. Most people regard National Wildlife Refuges as pristine areas reserved for wildlife and fish. However, during the World War II Aleutian campaign, Attu Island was of strategic importance and was occupied by both Japanese and American forces. Remains of buildings, tent camps, gunneries, fuel storage tanks, dumps, ammunition piles, utilities, and much ordnance are found on Attu. These lands are part of the Alaska Maritime National Wildlife Refuge. Attu is the westernmost of the Near Island group of the Aleutian Islands. It is over a thousand miles from the Alaskan mainland and 750 miles northeast of the northernmost of the Japanese Kurile Islands. Attu encompasses a variety of habitats including rolling tundra where cackling geese and rock ptarmigan nest, coastal cliffs that serve as nesting sites for thousands of seabirds, rivers where salmon spawn, and rocky shorelines where common eiders nest and feed offshore along with sea otters that are found in the coastal kelp forest. The majority of contaminant problems on Attu are from WWII activities. Leakage and spills from petroleum product tank farms and other storage areas, fuel lines, burn pits and barrel dumps are common, as are battery dumps, ammunition piles, and debris piles. Extensive soil and water sampling has been conducted at both islands by contractors for the US Army Corps of Engineers, the branch of the US Army responsible for assessing extent of contamination at former military sites. Contaminant analysis has also been done on some fish samples, soil, and sediment by USFWS. There has been no hazardous waste cleanup on Attu, with the exception of a large ordnance dump. Because some of the contaminated sites are due to long-term fuel leaks, these areas are a hazard to terrestrial birds that forage in these upland locations. There are also sites where during warm weather periods, fuel flows into nearby streams. In addition, groundwater contamination by petroleum hydrocarbons may also be a problem at tank farm sites. We are collecting and summarizing all available contaminant information on Attu for the US Fish and Wildlife Service Contaminant Assessment Process. This information will be used by the USFWS to make informed management decisions about contaminant threats and assist with site prioritization for Army Corps of Engineers cleanup actions.

**613 The Modified Guideline Study: What Can We Learn?** A. Samel, DuPont Crop Protection, Ecotoxicology. Standardized study designs have been developed by many organizations including the USEPA, the Organization for Economic Co-operation and Development (OECD), Environment Canada, ASTM and ISO. Standardized toxicity testing is an essential part of the regulatory process and often results in a collection of base-line data that is critical for the regulatory review of new and existing chemicals. The goal of standardized testing is to generate high quality results and reduce variability while increasing comparability across multiple data-sets. In a standardized test, the study method is defined using established protocols, regardless of the testing laboratory or the chemical tested, with similarly derived study end-points (EC/LC50, NOEC). Large data-sets of these similarly derived end-points provide a powerful tool to regulatory reviewers and investigators. The advantages of standardized testing, however, are also its biggest disadvantages. The standardized test represents a worst case scenario

that is often not representative of actual field conditions. For example, the maintenance of test concentrations for the duration of a standardized test in a water-only test system is often not representative of actual exposure duration times and chemical concentration levels in the field. In standardized tests, a single surrogate test species of uniform size and age is used to represent an entire taxonomic grouping (invertebrates, fish, algae). Modifications to standardized tests can increase the understanding of the fate and effects of a chemical. These modifications can range from testing non-guideline ecologically or regionally relevant test organisms to specific study design modifications that address, for example, pulse exposures with spikes and declines in concentration levels over time. Modifications might address specific questions such as the effects of fed versus unfed test organisms, effects to multiple age classes, testing in the presence of sediment, etc. Overall, modifications to standardized tests can provide a more comprehensive understanding of the fate and effects of a chemical to specific taxa and can provide information of greater ecological relevance without the need for field testing. Case studies of modifications to the standardized acute invertebrate toxicity test will be presented as examples of the usefulness and importance of the modified standard toxicity test.

**614 Control Treatment Water Selection Can Affect the Findings of Toxicity for *Selenastrum capricornutum* Toxicity Tests: A Source of False Positives?** S. Clark, R. Ogle, J. Cotsifas, Pacific EcoRisk. The USEPA has well-vetted and standardized aquatic toxicity test protocols that are applied nationwide in the determination of compliance with a wide variety of regulatory programs. Although there have been and continue to be efforts to further standardize test methods and reduce variability, the freshwater algae test with the green alga *Selenastrum capricornutum* allows the testing laboratory the flexibility to select different control/dilution waters for the tests (e.g., culture medium, uncontaminated receiving water, synthetic (reconstituted) water, or some other natural water). Several split-lab studies were performed in which the testing labs used different control waters, and the test results differed significantly as to identifying the samples as toxic or not. We hypothesized that in addition to the nutrients that are added per the test protocol, there can be significant differences in nutrients (and nutrient availability) in the different control/dilution water matrices used by different labs. This has the potential to result in biostimulation in some waters that could lead to a false conclusion of toxicity when compared to algal growth in an effluent. We performed experiments using several of the standard laboratory control waters used by commercial labs, with the results being compared to algal growth in actual test samples. The samples were identified as being toxic for some of the control waters, and not toxic for others, potentially resulting in "false positives" depending upon which control water was used for the regulatory compliance reporting. Results of these comparative tests will be discussed regarding implications for regulatory compliance.

**615 From Bugs in Mud to Fish in Coldwaters: Lessons Learned Adapting Standard Test Methods to Novel Conditions** J. Van Geest, Univ of Guelph, School of Environmental Sciences, Intrinsic Environmental Sciences Inc; D. Moore, Univ of Guelph, School of Environmental Sciences. Why start from scratch when so much work has already gone into the development and standardization of test methods? Designed for defined and general use, standard test methods are not always best suited to answer specific research and regulatory questions but are useful as a basis for the technical, quality control, and reporting aspects that are important to any study. Modifying certain aspects of standard methods is a means of rapidly moving research forward by allowing critical evaluation of emergent issues while still relying on the basic foundation of the method, giving merit to the argument that standard tests are still relevant in the changing face of environmental risk assessment. As researchers using standard methods, we discuss the lessons learned from our research adapting existing bioaccumulation test methods to develop the Ontario Ministry of the Environment's new bioaccumulation test method and assessing the impact of modifying factors on the existing Environment Canada rainbow trout standard test method. We provide an overview of the benefit and complications of adapting existing standardized test methods to answer new questions, important data gaps observed in the literature, and highlights of data obtained by modifying the standard test methods.

**616 Is *Hyalella* Reproduction a Practical Endpoint to Use in Toxicity Testing?** A. Bartlett, L. Brown, Environment Canada. Reproduction in *Hyalella* can be a more sensitive and environmentally relevant endpoint

than survival or growth; however, it is also much more variable, making it difficult to interpret the results and bringing into question the practicality of conducting reproduction tests on a routine basis. Previous research in our laboratory adapted standard sediment toxicity test methods for survival and growth to include a reproduction endpoint, and although reproduction appeared to be a more sensitive endpoint than survival, the data were too variable to use on a routine basis. The objective of this study was to conduct static-renewal sediment toxicity tests under control conditions (Lake Ontario culture water, Lake Erie sediment) to characterize reproduction in *Hyalella*, and then to manipulate test parameters, such as test duration, frequency of renewal, sediment:water ratio, and number and density of *Hyalella* in order to design methods to reduce variability in reproduction. The first set of experiments was conducted in 250-mL glass beakers with a 9:1 ratio of sediment:water and 10 replicates per treatment. In treatment 1, reproduction was characterized over 20 weeks by conducting static-renewal tests in which survival, growth, and reproduction were measured every 2 weeks starting at week 4. The juvenile:adult ratio was stable from 6-20 weeks, reproduction was highly variable (coefficients of variation ranged from 43-110%), and longer exposures did not reduce the variability in reproduction. Next, test duration and frequency of renewal were investigated by conducting: a static-renewal 10-week test with a renewal at week 4 (treatment 2), a static 6-week test (treatment 3), and a static 10-week test (treatment 4). When comparing the results to treatment 1: treatment 2 had a higher juvenile:adult ratio, perhaps due to reduced handling stress (one renewal versus three); treatment 3 was comparable; and treatment 4 had more variable (and lower) survival, likely related to deterioration of water quality. A second set of experiments is currently being conducted in 1-L Imhoff settling cones to stabilize overlying water quality. Test parameters, including sediment:water ratio and number and density of *Hyalella*, are being varied. The results of these tests will be presented and compared to those from 250-mL beakers. The practicality of using *Hyalella* reproduction as an endpoint in toxicity testing and directions for future research will be discussed.

**617 Minefields Associated with Mining Data from Peer-reviewed Literature** C. Russom, USEPA, Mid-Continent Ecology Division.

The USEPA's ECOTOX database is the largest compilation of ecotoxicity study results, providing information on the adverse effects of single chemical stressors to ecologically relevant aquatic and terrestrial species. The primary source of data included in the ECOTOX database is the peer-reviewed literature, with information gathering accomplished through manual data abstraction and data entry. ECOTOX is used by all sectors of the environmental toxicology community in risk assessments, screening and prioritization exercises, data analysis projects, and species and chemical extrapolation model development. Typically an evaluation of the relevance, adequacy, and reliability of a test result is conducted prior to using it in risk assessment and modeling efforts. Therefore, critical to the usability of this data is the complete reporting of experimental design and test results by authors. The ECOTOX database is designed to capture, in discrete data fields, all aspects of test methods and associated results, but assumptions about test protocols, and interpretations of the data are not made. Therefore many data fields remain unpopulated due to lack of detail provided in the publication. This presentation will cover limitations and information gaps related to published research results; how these information gaps may affect the inclusion of a study in products of research and in risk assessments (e.g., models, Agency benchmarks, etc.); and possible solutions such as open access data repositories. The contents of this abstract neither constitute nor reflect official USEPA policy.

**618 The Advantages and Application of Using a Light Dusting of Substrate for Experiments with Newly Hatched and 3<sup>rd</sup> Instar *Chironomus dilutus*** T.W. Valenti, National Research Council; US Environmental Protection Agency, National Research Council; T. Highland, R. Hockett, US Environmental Protection Agency, Mid-Continent Ecology Division; T.J. Norberg-King, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; D.R. Mount, US Environmental Protection Agency, ORD.

Ongoing research efforts are focused on identifying the relative degree of sensitivity provided by the standard 10-d sediment toxicity test initiated with 3<sup>rd</sup> instar *Chironomus dilutus* (common name: midge) compared to the modified 20-d sediment toxicity test initiated with < 1 h old newly hatched midges. The 10-d method is extensively used as an assessment tool because of its relative ease and low cost to conduct. The 20-d procedure is desirable because it is presumed to be more sensitive, but some laboratories have reported

difficulties achieving consistent results with the method. Prior comparisons suggest that earlier midge instars are more sensitive; however, most of these are based on acute exposures in which only lethality was monitored and it is unclear whether these differences will persist in longer exposures when sub-lethal endpoints are also measured. The overall goals of this research are to 1) better understand the relative sensitivity of 10-d and 20-d exposure methods and 2) determine if there are procedural changes that may improve laboratory success and consistency with the 20-d method. A major challenge for comparing responses between the methods is the inability to follow early survival and development of < 1 h old midges, as once they are added to sediment, it is nearly impossible to isolate them again from substrate until late in the test. To avoid this problem, we evaluated an alternative approach in which a light dusting of substrate is used. This approach provides organisms with materials to construct cases and allows easy recovery of animals for intermediate survival and growth measures. While a dusting of substrate does not allow for evaluations of bedded sediments, it can be used for water column exposures to directly compare the sensitivity of different life stages to toxicants. By selecting substrates that minimally interact with contaminants, it is possible to maintain consistent exposure concentrations and therefore accurately quantify the relative sensitivities across life stages of *C. dilutus* and between endpoints generated by the 10-d and 20-d procedures. High rates of survival and growth are shown for both < 1 h and 3<sup>rd</sup> instar old midges in both natural and artificial substrates under control conditions. In addition, we shall compare the responses observed during water-only exposure to copper, with emphasis on the relative sensitivity of each life stage. This abstract does not necessarily reflect USEPA policy.

**619 The Application of Multi-generation Tests in Aquatic Toxicology Research** P. Sibley, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Dept of Environmental Biology.

Benthic organisms in most contaminated freshwater environments are exposed to low concentrations of contaminants over relatively long periods of time. Realistic assessment of potential chronic effects in such cases is arguably best achieved using exposure regimes commensurate with these conditions. Most current standard test methods for long-term exposures typically encompass all or a portion of an organism's life cycle, as exemplified by the standard sediment tests for *Chironomus* spp. and *Hyalella azteca*, and the aquatic test using *Daphnia magna*. Life cycle tests can provide a wealth of information based on ecologically relevant endpoints such as growth and reproduction but are somewhat constrained in their capacity to extrapolate to populations, the protection of which is a key goal in ecological risk assessment. An extension of the life cycle test is the multi-generation test. Multi-generation tests use repeated applications of standard life cycle tests to investigate effects (e.g., enhanced tolerance/resistance, extinction, compromised immunity, etc) that are more likely to, or may only, become manifest after trans-generational exposure to contaminants. Using data generated from life cycle and multi-generational tests conducted with *C. dilutus*, *H. azteca*, and *D. magna* this paper will discuss the utility and challenges of developing and applying multigenerational tests in aquatic toxicology research. The potential value of multigenerational tests as a research tool to address questions that cannot be addressed using currently available standard life cycle tests, and the potential for developing new, or refining existing, standardized test methods will be discussed.

**620 What Makes Standard Toxicity Tests Tick?** R. Scroggins, Environment Canada, Ecotoxicology and Wildlife Health Division; L. Taylor, Environment Canada.

Biological toxicity tests have come a long way from ten fish in a bucket. Typical standard methods contain background information, guidance and specific procedures for culturing or holding of test organisms, test conditions, setting up a test, observations during the test, test take-down, analysis of results, and reporting. As an introduction to the session, this talk will provide an overview of the existing standardized test methods available and the value of following these methods in more research-oriented environmental assessments. Attendees will benefit greatly from learning about the advantages and limitations of the existing international and national standard methods, future plans to update older methods or develop new methods and the research that supports methodological improvement, and some of the challenges for future method development. In the application of standardized toxicity tests to research-type studies, we stress the importance of quality control and assurance practices which are relevant to all aspects of the test method (e.g., organism health criteria, allowed ranges of physical conditions such as temperature or lighting,



randomization of test vessels, minimizing technician bias, proper statistical analyses, test validity criteria, and minimum reporting requirements).

**621 Comparative Nanogenotoxic Study of DNA Damage Between Prokaryotes and Eukaryotes** J. Lan, N. Gou, Northeastern Univ, Civil & Environmental Engineering; A. Gu, Northeastern Univ, Dept of Civil & Env Engineering, Biotechnology Initiative Program, Dept of Civil & Env Engineering, Biotechnology Initiative Program. Nanomaterials (NMs) have been reported to induce DNA damage, which can potentially lead to mutational heritable changes in organisms. However, little is known of their detailed mechanism of damage and repair pathways involved. In this study, we investigated and compared transcriptional level effect and gene expression changes associated with a number of specific DNA damage and repair pathways in both prokaryotic and eukaryotic cells, after exposure to a number of different NMs. Transcriptional effect in prokaryotic cells was evaluated by GFP-infused K12 *E. coli* library with genes of DNA damage and repair regulation system including SOS system. RT-qPCR was applied to assess the changes in specific genes indicative of certain DNA damage recognition and repair pathways for eukaryotic A549 cell line. In addition, cytotoxicity test and comet test were performed for confirmation and comparison. NMs evaluated include nTiO<sub>2</sub>-anatase, carbon black, SWCNT (single wall carbon nanotube) and fullerene. MMC (mitomycin C), a model genotoxicant, was used as a positive control. The results showed that NMs leading to DNA damage to prokaryotic cells also caused DNA damage in eukaryotic cells, although detailed mechanism vary between species. Gene expression exhibited significant changes within 4 hours at doses lower than LD50, suggesting this method is more rapid and sensitive for sub-cytotoxic exposures. Among the NMs tested, nTiO<sub>2</sub> displayed the most wide range of genotoxicity for DNA damage, including single and double strand damage, as well as oxidative damage on bases. CB seemed to lead to different type and more severe DNA damage in eukaryotic cells. SWCNT and fullerene induced altered expression in genes involved in DNA damage and repair for eukaryotic cells but not in prokaryotes, suggesting that eukaryotic cells are more sensitive towards NMs genotoxicity, especially to more severe double strand break, likely due to the differences in their cell wall/membrane structure and therefore different susceptibility to DNA damages, directly or indirectly. Temporal trends of gene expression levels for A549 cells revealed details of the nature of DNA damage and repair response and, in comparison with parallel comet tests, indicated the variation in DNA damage generation, repair response and final DNA damage. The results demonstrated that gene expression may lead to a better understanding of damage mechanisms, which will help designing more benign NMs for wide applications.

**623 Size, Shape, and Surface Chemistry: The Three S's of Nanoparticle-Cell Interactions** T.R. Garner, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology; W.S. Baldwin, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX); A.M. Rao, Clemson Univ, Dept of Physics and Astronomy; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology (ENTOX). There are currently over 1000 consumer products on the market that contain or utilize nanomaterials, and this number is expected to increase exponentially in the near future in the absence of detailed knowledge concerning the interactions of such materials with biological systems. While nanoparticles have been shown to cross cellular membranes, little research has examined the influence of particle characteristics on membrane transport. Furthermore, our inadequate understanding of potential human and ecological effects has resulted in uncertainties of the potential risks of this technology and impeded the advancement of quantitative risk assessments. The goal this project was to characterize the influence of particle size, shape, and surface chemistry on the movement of gold nanoparticles across mammalian cellular membranes. A549 carcinomic human alveolar cells were utilized to characterize the movements of gold nanoparticles across cellular membranes. Specifically, the absorption of three different gold nanoparticle shapes were examined at multiple sizes: spheres (4, 10, and 50 nm), cubes (50 and 75 nm) and rods (20x100 and 20x200 nm). The influence of surface chemistry on absorption was examined through surface modifications of these particles of various shapes and sizes by protonated amine, PEG, and citrate. Each of these particles were characterized by TEM, DLS, UV-vis, and zeta potential. In total, comparisons of cellular interactions were examined for 21 different modifications of gold nanomaterials. Cells were

plated in 12-well plates at 100,000 cells per well and exposed to 1 mg/L gold for 0.5, 1, 1.5, and 2 hours. Bioaccumulation of nanoparticles at each time point was quantified by inductively coupled plasma-mass spectrometry (ICP-MS), and movement was visualized via confocal microscopy.

**624 Detection of Morphological Changes in *Pseudokirchneriella subcapitata* Following Exposure to Quantum Dots** J.L. Bouldin, Arkansas State Univ, Dept of Biological Sciences, Arkansas State Univ, Environmental Sciences Graduate Program, Environmental Sciences Graduate Program, Arkansas State Univ, Dept of Environmental Science; S. Yu, Arkansas State Univ, Arkansas Biosciences Institute. Semiconductor nanocrystals are presently used for research and diagnostic techniques in biological testing; increased applications of these nanoparticles for industrial and consumer products has increased global use with further increases predicted. An organic coating protects an inner core of cadmium and selenium in these fluorescent nanocrystals. Following Quantum dot (QD) exposure, microscopic results revealed a change in cellular shape to the single-celled alga, *Pseudokirchneriella subcapitata*. Changes in cell size were determined by polygon surface area measurements. Results reveal a statistically greater surface area in cells exposed to QDs when compared to unexposed cells. A 56% increase in cell surface was measured in algae exposed to 55 ppb Qdot suspensions. Cellular changes were further quantified using a scanning electron microscope and flow cytometry. Flow cytometry analyses reveals changes in algal cell populations with increased surface area and granularity following 72- and 96-hr exposures and fluorescein diacetate (FDA) was used to determine cell-membrane integrity. Scanning electron microscopy was used to determine changes in mean length and width of individual exposed algal cells. Although outer layers offer a protective coating from the toxic metals present in the QD core to multi-cellular organisms, toxicity to single-cell algae poses a concern for environmental protection from nanoparticle release.

**625 Alteration of Biochemical Pools in Cyanobacteria Induced by Nano-titanium Dioxide Exposure** C. Cherchi, Northeastern Univ, Civil and Environmental Engineering; A. GU, Northeastern Univ, Dept of Civil & Env Engineering, Biotechnology Initiative Program, Dept of Civil & Env Engineering, Biotechnology Initiative Program. Progress of nanoscience poses an urgent need for fundamental understanding of the potential environmental effects of engineered nanomaterials such as nano-titanium dioxide (nTiO<sub>2</sub>), whose presence in wastewater effluents have already been detected and will likely impact aquatic systems (e.g., algae). In this study Fourier Transform Infrared spectroscopy (FT-IR) was used to evaluate the impact of nTiO<sub>2</sub> at environmentally relevant doses on the cellular reorganization of macromolecules in the cyanobacteria *A. variabilis*. The comparison of the predominant chemical markers (proteins, carbohydrates, lipids, nucleic acids) obtained showed characteristic temporal changes during the short-term (96 hrs) and long-term (21 days) exposure to nTiO<sub>2</sub>. Most dynamic variations, as indicated by the changes in the ratios of macromolecules to proteins, were observed during the first 96 hrs and mostly showed a dose-dependent pattern; however, over longer time (21 days), the cells seemed to be able to recover back to more conservative levels of macromolecule composition and, the rate and time required for the recovery as well as the final ratios of the macromolecules varied with nTiO<sub>2</sub> doses. For example, nTiO<sub>2</sub> exposure led to a sudden increase in proteins levels after the initial dose (within 3 hrs) then followed by protein reduction (following 96 hrs), and the magnitude of initial protein increase and the subsequent reduction rates were found to be dose-dependent. Changes in the amide II/amide I ratio and in the -CH<sub>2</sub> frequencies of lipids revealed that conformational changes in membrane protein folding and cells' structural changes are possibly induced by nTiO<sub>2</sub> treatment, respectively. Temporal variations in the phosphodiester stretchings suggested that changes in the relative content of nucleic acids were likely induced by nTiO<sub>2</sub>, and might impair the biogenesis of ribosome, a significant repository of phosphorus in ecosystems. Principal component analysis of FT-IR spectra showed that specific functional groups (i.e., carbohydrates) contribute to the differences among treatments. The results clearly indicated that an array of responsive changes were caused by nTiO<sub>2</sub>, targeting membrane components, proteins and RNA-associated functional groups, energy storage products and the depository of genetic information. In conclusion, this study provides insights into the potential larger scale impact of nTiO<sub>2</sub> on ecological interactions and food web dynamics in aquatic ecosystems.

**626 Toxicogenomic Evaluation of the Single-Walled Carbon NanoTube (SWCNT) Electronic Structure-dependent Interactions with Microorganisms** N. Gou, Northeastern Univ, Civil & Environmental Engineering; C.Z. Vecities, Harvard Univ, School of Engineering and Applied Sciences; A. Gu, Northeastern Univ, Dept of Civil & Env Engineering, Biotechnology Initiative Program, Dept of Civil & Env Engineering, Biotechnology Initiative Program. Previous SWCNT toxicity studies have primarily focused on the relative toxicity as a function of physicochemical properties including diameter, surface functionality, and their subsequent dispersion/aggregation state. Another important physicochemical property that has been shown to affect the reactivity of SWCNT is its chirality-dependent electronic structure, i.e., whether the SWCNTs are metallic or semiconducting. In this study, we applied a toxicogenomic approach towards the mechanistic assessment of three purified and well-characterized SWCNTs of similar diameter and length, but consisting of varying fractions (< 5, ~30, and >95%) of metallic SWCNTs referred as S-SWCNT, X-SWCNT, and M-SWCNT, respectively. A whole-cell-array library of recombinated *E. coli* K12 strains with promoter-reporter GFP fusions the majority of known stress response genes is utilized to examine the relative toxicogenomic affects of these three SWCNT samples. A recently developed Transcriptional Effect Level Index (TELI) is used to quantify the results. The stress library TELI results indicated that M-SWCNTs induced a higher response level than the M-SWCNT and the S-SWCNT. In all cases, SWCNT exposure significantly affected the transcription level for oxidative stress and membrane stress genes suggesting these to be the two major toxicity pathways. The metallic SWCNTs induced significantly higher transcriptional level changes in oxidative stress (6 fold) and membrane stress (2.5 fold) than the semiconducting SWCNTs. Specifically, for sub-categorized membrane stress functions, metallic SWCNTs induced higher altered gene expression of those involved in electron transfer, membrane structure related macromolecular metabolism, flagellar biosynthesis, and antibiotic resistance. These results indicated that there is clearly an electronic-structure-dependent reactivity of SWCNTs and therefore varying toxicity level, consistent with the previous report. The information obtained in this study revealed details of the cellular response to these two SWCNT and provided insights into the correlation of electronic properties of SWCNT with its toxicity. The significantly higher stress response caused by the metallic SWCNT than the semiconducting SWCNT seemed to suggest that the toxicity level and mechanism of SWCNT are related with the electronic structure and electron transfer ability of the SWCNT.

**627 Influence of Nanosilver on Bacterial Diversity in Natural Waters** P. Das, Trent Univ; C.J. Williams, Trent Univ, Dept of Biology; R.R. Fult-horpe, Univ of Toronto, Dept of Physical and Environmental Sciences; M.E. Hoque, C.D. Metcalfe, Trent Univ, Environmental and Resource Studies; M.A. Xenopoulos, Trent Univ, Dept of Biology. The widespread usage of nanosilver (nAg) due to their antibacterial properties possesses a potential environmental risk once they are released into aquatic environment. Many studies have investigated the effects of nAg on various aquatic organisms, but very little is known about their possible toxicological effects on bacterial community composition in natural aquatic systems. We investigated the effects of nAg on bacterial community composition collected from nearby aquatic ecosystems and exposed to several nominal concentrations of nAg (ViveNano Inc.; 10nm particle size) at a level of 0.01 - 1 mg Ag L<sup>-1</sup> for 5 days. We found inhibition of bacterial growth in most nAg treatments just after 1 hour, but at low nominal concentrations of 0.05 to 0.5 mg L

**628 Impact of Nanoparticles to Soil Microbial Community Under Field Conditions** V. Shah, Dowling College; D. Collins, Dowling College, Dept of Biology; V. Walker, N. Kumar, Queens Univ, Dept of Biology; T. Luxton, US Environmental Protection Agency. In recent times, impact of nanoparticle on soil microbial community has been discussed in the literature and change in the community structure observed. However, all such studies have been carried out in some form of controlled environment. In the study to be presented, we will illustrate the fate of copper and zinc oxide nanoparticles in soil under field conditions and show its impact on soil microbial community. Nanoparticles were sprinkled on the surface of the soil and let to stand for six months. Transport of the particles within the soil along with speciation changes on the nanoparticles were monitored as a function of time along with the changes in microbial community. Changes in microbial community were also monitored using multiple methods such as pyrosequencing, fatty acid methyl ester extraction method, and community level physiological profiling using Biolog® ecoplates.

**629 Conserved Developmental Pathways and Disease Processes: Implications from Genomics Datasets Across Species** J.M. Gohlke, Univ of Alabama at Birmingham, Dept of Environmental Health Sciences. Pathway or network-based methods are commonly used in the analysis of genomics datasets to provide insight into the biological processes potentially involved in the human or ecological endpoints under study. Pathway-based methods usually refer to analysis using interactions between gene products based on multiple sources of evidence from previous research published in the literature, whereas network-based approaches may also rely on de novo interaction predictions from genomics datasets. Several lines of evidence suggest conserved developmental pathways or networks may offer a useful framework for cross-species analysis. A pathway-based method describing analysis of genomics datasets to develop an environmental factor-disease network to identify and prioritize key pathways for human health relevance will be presented. In addition, a gene regulatory network describing cell fate specification in the developing mammalian forebrain and the impacts of TCDD will be presented. The relevance of these approaches in bridging ecological and human health evaluations will be evaluated, with a particular focus on phenotypic outcomes across species. As an example, the Notch pathway is a highly conserved developmental pathway that regulates cell fate specification, and therefore cell number and differentiation status across spatially and temporally restricted gradients in invertebrate and vertebrate models. Recent analyses in zebrafish and *Drosophila* link Notch signaling to regulation of dopaminergic neuronal specification, reproduction, and pigmentation phenotypes. Several human diseases are now understood as being mediated via the Notch pathway and this has guided the development of Notch based high throughput assays, such as the quantification of pigmentation phenotypes in zebrafish embryos as a screen for gamma-secretase inhibitors. Hence, highly conserved developmental pathways may provide a useful construct for integrating genomic evidence across human and ecologically relevant species in support of human and ecological risk assessment, and co-option of conserved pathways for different functions across species is highlighted as an important area for future research.

**630 Genetic Architecture of Evolved Tolerance to PCBs in the Estuarine Fish *Fundulus heteroclitus*** E.R. Waits, USEPA, NERL; S. Morris, B. Rinner, USEPA; D. Proestou, US Environmental Protection Agency, Atlantic Ecology Division; D. Champlin, USEPA; D. Nacci, USEPA, ORD, NHEERL, Atlantic Ecology Division, USEPA, NHEERL, Atlantic Ecology Division. Populations of Atlantic killifish (*F. heteroclitus*) resident to coastal estuarine habitats contaminated with halogenated aromatic hydrocarbons (HAHs) exhibit heritable resistance to the early life-stage toxicity associated with these compounds. Beyond our knowledge of the aryl hydrocarbon receptor (AHR) and its role in mediating this toxicity, little else is known concerning the precise downstream targets responsible for this adaptation. In the present study, we performed a genome-wide survey for quantitative genetic variation among differentially sensitive killifish in order to elucidate the genetic basis of evolved tolerance. Following exposure to PCB126, the recombinant generation of a genetic cross between PCB-sensitive (Block Island, RI) and PCB-tolerant (New Bedford, MA) killifish were phenotyped to determine sensitivity and genotyped with a dense panel of co-dominant molecular markers distributed across the genome. Multiple loci were identified strongly associated with PCB tolerance-some acting alone and others via additive and epistatic interactions. Together, these loci account for at least 44% of the phenotypic variance observed in susceptibility following PCB126 exposure between Block Island and New Bedford killifish (LOD = 11.67,  $P = 1.14 \times 10^{-5}$ ). These data demonstrate that PCB126-induced developmental toxicity is a complex trait influenced by gene x gene and gene x environment interactions, and provide insight to the evolutionary basis of evolved tolerance to chemical contaminants in wild populations.

**631 Genetic Variation Between Clones of *Folsomia candida* (Collembola) is Associated with Differential Fitness – and Transcriptional Responses to Cadmium** B. Noto, VU Univ Amsterdam, Animal Ecology; D. Roelofs, VU Univ Amsterdam. The parthenogenetic species *Folsomia candida* (springtail) is frequently used as a standard test organism for soil quality assessment. Different genotypes (clonal lines), identified with both mitochondrial and nuclear markers, showed different life history trait responses to the soil-pollutant cadmium. Animals with a certain genotype seemed more tolerant to cadmium toxicity than animals with another. Microarray gene expression analysis was used to gain insight in the biological processes and mechanisms responsible for (or effected by) the different

response to cadmium. Genotype-specific changes in the transcriptome in response to cadmium revealed that fewer genes are differentially expressed in tolerant animals when compared to sensitive ones (223 and 1025 genes, respectively). A 2x2 factorial analysis (limma) identified 97 genes that showed an interaction between genotype and cadmium treatment (BH adjusted  $p < 0.05$ ). The expression of these genes in response to cadmium showed a much more conserved pattern (i.e., lower or no fold changes) in the tolerant animals compared to the sensitive ones. Furthermore, the expression of these genes showed much more correlation with each other in sensitive animals. Many of these genes are involved in oxidation/reduction-like processes. The array data suggest that cadmium sensitive animals, when exposed to cadmium, show a typical transcriptional signature associated with stress, while the tolerant animals maintain 'normal' expression for most of their genes. These findings will have consequences for the use of genomic tools in ecotoxicology and environmental quality assessment. This study also provides insight in molecular processes behind tolerance associated with different genotypes, and suggests new evolutionary mechanisms involved in parthenogenesis.

### 632 Heritability of the Reproductive Response in Adult Fathead Minnow Exposed to an Anti-androgen K.L. Thorpe, Canadian Rivers Institute, Univ of Prince Edward Island, Dept of Biology; E. Postma, Univ of Zurich-Irchel, Institute of Evolutionary Biology and Environmental Studies; M. Koelliker, Univ of Basel, Zoologisches Institute. Endocrine disrupting chemicals are a globally important group of environmental contaminants known to affect populations through skewing sex ratios and inhibiting reproduction. However, little is known about their potential to influence evolutionary processes. In part, this is because most experiments have focused primarily on determining functional responses in the average animal of the group and have failed to consider the importance of individual variation in responses to exposure. Such approaches ignore the fact that variation in phenotypic traits is an essential feature of populations as, if heritable, it provides the basis for populations to evolve and adapt through the process of selection. In this study, between-pair variation in the sensitivity of reproductively active fathead minnow exposed to the anti-androgen flutamide (400 µg/L) was quantified and heritability of the observed variation determined. As expected, flutamide exposure reduced mean reproductive success (number of viable offspring) by 56%. Furthermore, there were not only large differences in reproductive success among pairs, but also in how strongly they were affected; reproduction was completely inhibited in 27% of the exposed pairs but unaffected in 23% of pairs. Although this suggests that flutamide exposure may generate selection for lower sensitivity in natural populations, the evolutionary response to selection depends on whether there is any heritable variation. Non-exposed offspring were, therefore, collected from each FHM pair, raised until sexually mature, and males and females from the different lines crossed to assess reproduction in the absence/presence of flutamide. Quantitative genetic mixed model analyses showed a substantial heritable component to variation in mean reproductive success (i.e., between treatments) as well as some heritable variation in flutamide sensitivity, indicating that both could respond to selection. There was also a strong positive genetic correlation between the two, with pairs of high average fitness being less sensitive to flutamide, suggesting there is no trade-off between reproductive success and flutamide sensitivity. Based on these findings, the potential for exposure to anti-androgens to modify evolutionary processes in natural fish populations will be discussed.

**633 Meta-analysis of Toxicogenomic Data from Multiple Species Reveals Conserved Responses Across Divergent Phylogenetic Lineages** K.A. Gust, US Army, Engineer Research and Development Center, Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, ERDC-EL-EP-P; N. Garcia-Reyero, Jackson State Univ; T. Habib, BTS; M. Pirooznia, The Johns Hopkins Univ, School of Medicine; P. Gong, SpecPro Inc., Environmental Services; C. Warner, The Keck Institute; M. Wilbanks, US Army, Engineer Research and Development Center; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division. A principle task of our research laboratory is to holistically assess the impacts of environmental contaminants, principally munitions constituents (MCs), on military ranges. A great deal of research has been conducted to describe the impacts of the MC hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). RDX has been shown to impact the central nervous system causing seizures in humans and other higher vertebrates. Comparative toxicology

and comparative genomics can be used to assess the effects of a contaminant among species. For this effort, we analyzed the effects of RDX on five species to elucidate if the compound elicits effects via common toxicological mechanisms among the species examined. Our approach included phylogenetic analysis of GABAA (the substrate to which RDX binds), gene set enrichment analysis (GSEA), and context likelihood of relatedness analysis to infer transcriptional networks to compare and contrast the neurotoxic effects of RDX among five phylogenetically disparate species: rat (Sprague Dawley), the fathead minnow (*Pimephales promelas*), earthworm (*Eisenia fetida*), Northern bobwhite (*Colinus virginianus*), and coral (*Acropora formosa*). Our results showed that RDX accumulated in coral and earthworms and into the brain of rat, Northern bobwhite and the fathead minnows. Phylogenetic comparison indicated that the molecular initiating event for RDX toxicity (GABAAR-binding) was conserved across all species. The comparison of Gene Ontology terms indicated several biological processes affected by RDX in all species, such as impacts on calcium signaling (involved in seizure response), effects on xenobiotic metabolism, electron transport, and cell signaling pathways. Metabolic pathway and transcriptional network analysis of RDX impacts across species demonstrated that responses to RDX exposure are highly conserved within closely related species. However, as evolutionary distance increased, effects related to the molecular initiating event (RDX binding to GABAAR) decreased and more general effects on metabolic functions became prevalent.

### 634 mRNA and microRNA Transcript Profiling in Atlantic Killifish (*Fundulus heteroclitus*) Embryos from PCB-sensitive and PCB-resistant Populations S.I. Karchner, Woods Hole Oceanographic Institution, Biology; N. Aluru, Woods Hole Oceanographic Institution; M.J. Jenny, Univ of Alabama; D.B. Mark Welch, Marine Biological Laboratory; M.E. Hahn, Woods Hole Oceanographic Institution. The Atlantic killifish is a valuable model for investigating evolutionary adaptations to chemical exposure. Killifish inhabiting the PCB-contaminated Superfund site in New Bedford Harbor, MA (NBH) have developed resistance to embryotoxicity and activation of the aryl hydrocarbon receptor (AHR) signaling pathway after exposure to PCBs and other AHR agonists. A lack of genomic information has limited efforts to understand the molecular mechanisms underlying the PCB resistance of NBH fish. The advent of high throughput sequencing has facilitated an assessment of mRNA and microRNA transcriptomes and changes associated with the evolved resistance in killifish. In this study, transcript profiling by deep sequencing was performed on PCB-exposed killifish embryos from NBH and a reference site, Scorton Creek, MA (SC). Non-normalized, 3'-anchored EST libraries yielded ~175,000 reads from each library. Shotgun sequencing on 1-15 dpf pooled samples of NBH and SC killifish embryos provided broader sequence coverage (~1,500,000 reads) and facilitated annotation of the ESTs. Results indicate several novel PCB-induced and repressed genes in both populations, with limited overlap between the populations. We also profiled microRNAs in these embryos using SOLiD sequencing. Embryos from NBH and SC were collected daily from 1 to 15 days post-fertilization (dpf) and pooled samples from each site were used for deep sequencing. SOLiD sequencing resulted in 2.5 and 2.0 million trimmed reads from the SC and NBH samples, respectively. Out of the 358 total number of known zebrafish microRNAs, 342 were identified among the killifish sequences. The unmatched reads were used in a search against the known human microRNAs, which resulted in the identification of 11 corresponding killifish sequences. Comparison of annotated microRNAs between NBH and SC is ongoing. [P42ES007381, R21ES017304]

**635 Understanding How *Daphnia* Populations Vary in Their Genomic Responses to Chemicals and Binary Mixtures** J.K. Colbourne, Indiana Univ, and Mount Desert Island Biological Laboratory, The Center for Genomics and Bioinformatics; J. Lopez, Indiana Univ, The Center for Genomics and Bioinformatics; S. Glaholt, Indiana Univ, School of Public and Environmental Affairs; M.E. Pfrender, Univ of Notre Dame, Dept of Biological Sciences; J.R. Shaw, Indiana Univ, and Mount Desert Island Biological Laboratory, School of Public and Environmental Affairs. *Daphnia*, or the water flea, is a sentinel species of freshwater ecosystems. Their populations are defined by the boundaries of ponds and lakes, are sensitive to modern toxicants in the environment, and thus are used to assess the ecological impact of environmental change. Their short generation time, large brood sizes, and ease of laboratory and field manipulation have assured *Daphnia's* importance for setting regulatory standards by environmental protection agencies, for testing chemical safety, for monitoring water quality,



and as a model for environmental genomics research. In this study, we take advantage of the animal's clonality and mature genomics tools to partition the sources of gene expression variation in the stress response of genetic isolates to three environmentally relevant pollutants, and their binary mixtures: Arsenic, Acetaminophen, Atrazine. One isolate originates from a natural population that has faced severe chemical challenges for over a century of industrial iron/ore smelting and thus demonstrates evolved tolerance to toxic levels of certain metals. Another isolate originates from a population that has no history of chemical stress. By interrogating differential expression of 31,000 annotated genes from 25 comparisons across conditions and between isolates, this study provides new insights into the functional interactions among genes and environment. The research was conducted during hands-on training in "Environmental Genomics" – an annual summer course offered at the Mount Desert Island Biological Laboratory. See [http://www.mdibl.org/courses/Environmental\\_Genomics/199/](http://www.mdibl.org/courses/Environmental_Genomics/199/).

**636 Using the Model Organism *Daphnia pulex* to Delineate Molecular Pathways Responding to Environmental Stress** K.A. De Schamphelaere,

Ghent Univ, Environmental Toxicology and Aquatic Ecology, Ghent. Univ (UGent), Environmental Toxicology and Aquatic Ecology, Ghent Univ, Environmental Toxicology and Aquatic Ecology; J. Asselman, D. Deconinck, UGent; S. Glaholt, Indiana Univ; C. Janssen, UGent; J. Colbourne, J. Shaw, Indiana Univ. Recently, the full genome sequence of *Daphnia pulex* has been published. Along with this, a suite of molecular tools dedicated to this emerging model species is becoming available, including a high-density (12x130k) whole-genome long oligonucleotide microarray for whole-genome expression studies. In this study we have used this microarray to study whole genome expression of two isolates of *Daphnia pulex* exposed to Cd and the toxic cyanobacteria *Microcystis aeruginosa*. An automated pathway analysis pipeline was developed and programmed in R and this was used to delineate molecular pathways responding to these environmental stressors. For instance, this analysis revealed that exposure to *M. aeruginosa* invoked major changes in oxidative phosphorylation, carbohydrate metabolism and protein turnover. Moreover, a comparative analysis of responses of two isolates of *D. pulex* (one tolerant to *Microcystis* and one sensitive to *microcystis* in terms of reproductive inhibition) is beginning to shed light on the molecular mechanisms involved in the genetic basis of large tolerance differences observed among individuals of this species. This research contributes to and benefits from the *Daphnia* Genomics Consortium.

**637 Prioritizing CECs for Aquatic Ecological Applications** J. Diamond,

H. Latimer, Tetra Tech, Inc.; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; J. Gilliam, Tetra Tech, Inc. With improved analytical detection capabilities, a variety of organic chemicals of emerging concern (CEC) have been found in trace amounts in surface waters, sediment, and fish tissue. These CECs include pharmaceuticals, personal care products, surfactants, and other currently unregulated chemicals. Identifying or predicting ecological effects of CECs in aquatic systems requires different tools that can diagnose effects at multiple scales of ecological organization. Developing an approach to prioritize CECs having the greatest potential risk to aquatic communities is one critical step that could help address this question. We developed three approaches to prioritize CECs: (1) hazard-based, (2) chemical persistence, bioaccumulation potential, and toxicity (PBT), and (3) a hybrid based on hazard, persistence, and bioaccumulation potential. Using an occurrence database compiled from over 100 monitoring studies, the three prioritization approaches were applied to over 500 CECs that have been measured in water or effluent samples in the US over the past 10 years. Types of CECs identified as high priority differed among approaches: steroids/hormones, pharmaceuticals, and surfactants comprised most of the high priority CECs based on hazard alone while pesticides, industrial chemicals, and PAHs (including many CECs that are currently regulated) comprised most of the high priority CECs based on a PBT approach. Except for the synthetic hormones and steroids, results of all three prioritization approaches yielded only a few pharmaceuticals of high priority. Using a hazard-based prioritization approach, predicted chronic toxicity endpoints were more sensitive than endpoints based on estrogenic activity for most CECs. The process of developing the list is as important as the list itself and appropriate use of any priority list will depend on the goals of the user. The hazard-based approach appears to be a useful starting point for monitoring, particularly for sites that are influenced by wastewater.

**638 Prioritizing Pharmaceuticals in Municipal Wastewater** M. Kostich,

USEPA, NERL, National Exposure Research Laboratory, USEPA; J. La-zorchak, A. Batt, S. Glassmeyer, R. Flick, J. Martinson, National Exposure Research Laboratory, USEPA. This talk will describe our prioritization of active pharmaceutical ingredients (APIs), based on estimates of risks posed by API residues originating from municipal wastewater. The goals of this project include prioritization of APIs for future research based on relative risks, and the estimation of absolute risks for individual APIs and their mixtures. The risk estimates were calculated as the ratio of an exposure rate estimate to an estimate of the lowest observable effect level (LOEL) for each API. The initial datasets used for exposure predictions included nationally aggregated drug sales and per capita wastewater production rates, which were combined in order to estimate wastewater influent concentrations. There are many shortcomings to these data, and no reliable estimates could be made for certain steps in the exposure model, most critically including in-plant and in-stream degradation rates. Several datasets were used to estimate the LOEL, including minimum therapeutic dose rates and plasma concentrations, both of which are typically available for this class of contaminants. The drawbacks and merits of these approaches will be discussed. For a handful of APIs, more detailed marketing data was available, allowing us to estimate site-to-site variability in exposure rates, which, in turn, provided the opportunity to evaluate our exposure predictions against actual measurement data. These results suggest our model is useful for estimating a ceiling for exposure rates for a particular API, but often underestimate typical exposure rates, likely due to overestimates of API persistence. The results of the prioritization will be briefly described, and compared to similar risk estimates we have made for endogenous human estrogens in wastewater.

**639 Prioritization of Human and Veterinary Pharmaceuticals for Ecological Risk Assessment – An Experience in Korea** K. Choi, Seoul

National Univ, School of Public Health, Seoul National Univ, Dept of Environ. Health; Y. Kim, Seoul National Univ; J. Park, Soonchunhyang Univ. Pharmaceutical residues may have impacts on nontarget biological organisms in aquatic ecosystems, and have therefore precipitated numerous investigations worldwide. Due to the great number of active pharmaceuticals which are available on the market, appropriate prioritization efforts are needed to identify the compounds that require environmental monitoring and ecotoxicological investigations. We prioritized both human antibiotics and veterinary pharmaceuticals in Korea by their usage, potential to enter the environment, or potential risk. For this purpose, the total amounts of active human pharmaceutical ingredients were estimated using the Korea Pharmaceutical Manufacturers Association (KPMA)'s monetary database. Expected introduction concentration (EIC) for each pharmaceutical was determined and corrected with excretion rate. Ten human antibiotic substances identified to have EICcorrected greater than 1  $\mu$ /L, include amoxicillin, cefaclor, roxithromycin, cephradine, cefatrizine, cefadroxil, aztreonam, ceftazidime, ribostamycin, and ceftazole. Among veterinary pharmaceuticals, twenty compounds were identified as top priority, most of which were antibiotics. Eight veterinary pharmaceuticals were considered to deserve more immediate attention: amoxicillin, enramycin, fenbendazole, florfenicol, ivermectin, oxytetracycline, tylosin, and virginiamycin. To further narrow the list of target compounds for ecological risk assessment, available ecotoxicological information was also considered. Some of these compounds have been investigated for ecological risks in Korean waters.

**640 Prioritization of Biosolids-borne Trace Organic Chemicals** C.

Higgins, Colorado School of Mines, Environmental Science and Engineering; D. McAvoy, Univ of Cincinnati; G. O'Connor, Univ of Florida. Trace organic chemicals (TORCs) present in municipal biosolids in the US have recently received considerable attention by the public and scientific community. Whether the presence of TORCs in biosolids results in significant risks to public health and the environment following land application is a question that remains at least partially unanswered. Though the US Environmental Protection Agency evaluated the risks associated with dioxins in biosolids-amended soils, this Water Environment Research Foundation (WERF)-sponsored effort sought to examine what data would be needed to conduct risk assessment for TORCs in biosolids-amended soils as well as for which TORCs these data would be needed. This present study included an evaluation and prioritization of TORCs of greatest concern in the terrestrial environment. The assessment and prioritization was based on occurrence data and readily available information on basic properties such as bioaccumulation and toxicity. The most important parameters for conducting

ecological risk assessments and the techniques currently available for obtaining the parameter values were also identified through an evaluation of quantitative risk assessments approaches, the end result being the identification of a minimum data set needed for risk modeling. For the TORCs identified as high priorities, a comprehensive literature review identified relevant data on fate, transport, biotransfer from soil to plants and animals, and toxicity in the terrestrial environment. From this review of the literature, data gaps were identified for the parameters most important for conducting terrestrial risk assessments.

**641 Prioritizing Persistent and Bioaccumulative Chemicals of Emerging Concern: Starting Materials, Impurities, By-products and Degradation Products** P.H. Howard, SRC, Inc.; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute. The Great Lakes and other North American regions have always been in the forefront in terms of identification of new environmental contaminants, particularly persistent (P) and bioaccumulative (B) organic chemicals. However, the identification has been by detailed analytical chemistry and is focused largely on structural analogs of previously identified compounds with only limited application of "in silico" approaches. Of particular concern are those substances that are P and B as defined by screening criteria for persistent organic pollutants (POPs). Such chemicals can move off shore from urban source regions and accumulate in waters and sediments of the open lakes and rivers where they can result in exposures of top predator fish and fish-eating birds. With improved instrumental capabilities, the past 10 years has seen a large number of new chemicals identified, mainly halogenated organics. However, the chemicals identified represent only a small fraction of the organic chemical substances in commerce. In a previous study, we have identified over 600 P&B chemicals using quantitative structure-activity relationships (QSARs) and expert judgment from a database of 22,000 commercial chemicals used in North America. In this presentation, we will examine starting materials, impurities, by-products, and degradation products/metabolites of the P&B commercial chemicals. Most commercial chemicals are synthesized from starting materials and reagents and result in the desired product plus leftover starting materials and by-products. These leftover starting materials or by-products can be environmental contaminants just like the desired commercial products. By reviewing the commercial synthetic pathways, we were able to identify possible leftover starting materials, impurities, and by-products. In addition, once a commercial chemical is used, it may be released to the environment in a variety of ways. Using available software and databases, we were able to predict potential degradation products/metabolites. Examples of impurities, by-products, and degradation products as well as some prediction successes (chemicals predicted to be P and B for which measurements has subsequently shown to be present) will be presented.

**642 Generation of 2D QSARs for Properties Relevant to Chemical Prioritization** T.N. Brown, Helmholtz Centre for Environmental Research UFZ, Dept of Analytical Environmental Chemistry; J.A. Arnot, F. Wania, Univ of Toronto Scarborough, Dept of Chemistry, Dept of Physical and Environmental Sciences; K. Goss, Helmholtz Centre for Environmental Research UFZ, Dept of Analytical Environmental Chemistry. In recent decades regulations and methods have been developed to evaluate tens of thousands of chemicals; however, there are few measured data available for the legislated assessments. In this context, mass balance and quantitative structure-activity (property) relationships (QSA(P)Rs) are needed for chemical screening and prioritization and for chemical exposure, hazard and risk assessments. Chemical partitioning properties and reaction half-lives are required for PBT assessments and for holistic multimedia models for exposure and risk assessment. The Iterative Fragment Selection (IFS) method has been developed to generate and evaluate QSA(P)Rs and to build knowledge infrastructure for chemical assessments. IFS is an integrated method of fragment generation, dataset splitting, and model selection. This QSA(P)R method does not require pre-existing expert knowledge on structural information related to a specific chemical property. 2D fragments are generated automatically from the dataset and the method iteratively selects fragments and QSARs through an automated process of fragment selection and model testing. The application of the IFS QSA(P)R development method is illustrated for properties relevant to chemical risk assessment; fish biotransformation half-lives, water solubility, and the octanol-water partition coefficient. Merits and limitations of the IFS method are discussed as are opportunities for

applying the QSA(P)R development and evaluation methods to other datasets to address pressing data gaps in chemical assessment programs.

**643 Experimental Design for Assessing the Potential Ecological Risk of Consumer Product Chemicals** S. Dyer, The Procter & Gamble Company, Central Product Safety, The Procter & Gamble Company, Miami Valley Innovation Center; K. Kapo, Montani Run, LLC. Receiving water biology is exposed to nutrients, metals, and organic chemicals from a myriad of sources, including storm water runoff, agricultural chemicals, industry, and down-the-drain disposal of pharmaceuticals and consumer products (often referred to as contaminants of emerging concern, CECs). Due to the myriad of sources, a pragmatic approach is required to reduce the potential confounding factors that relate CEC exposure and measured biological effects. In this presentation we propose, and demonstrate via case studies, an approach that integrates the use of modeled and measured data collected from industrial, state and federal sources to provide a suite of prioritization criteria used for site selection that minimizes confounding factors and provides a mechanism by which site based study results may be extrapolated to the thousands of river reaches that receive CECs via wastewater treatment discharge. The base for our approach is the national scale model iSTREEM (publically available via the American Cleaning Institute). The model utilizes wastewater treatment characteristics found within the USEPA's Clean Water Needs Survey, such as treatment type, dilution, population served and percent industry as key factors that can be queried to form a hierarchical understanding of existing discharge conditions. USEPA's Permit Compliance System provides information on the efficacy of historical treatment. Measured chemistry (conventional pollutants, nutrients) and instream habitat quality from Ohio EPA add instream relevance to the prioritization scheme. Finally, the relationships of all the above factors with the dependent variable, biological condition (invertebrate and fish community status), provides the means to obtain an ecological reality check for potential site selection. Since all these data and prioritization analyses are housed within a geographic information system, it is possible to ascertain potential sites where CECs may be related to biological impacts and, therefore, provide design criteria for subsequent site-based monitoring efforts.

**644 How to Prioritize?** T. Verslycke, M. Sharma, Gradient. If "everything is everywhere", how does one focus limited resources on those compounds that are most likely to be present at levels that warrant environmental or human health concerns? Definitions for "chemicals of emerging concern" remain fluid and numerous lists of such chemicals have been developed using a variety of approaches, ranging from intrinsic hazard to perceived risk, from measured environmental concentration to production volume. A better approach for defining "chemicals of emerging concern" would be to use a risk assessment framework, a methodology that has been implemented and used for decades to support, for instance, chemical and product safety assessments and market approval, site remediation, and water quality criteria setting. This talk will present such a framework, which could serve as the technical basis for regulatory prioritization efforts regarding environmental monitoring and assessment of chemicals. The presentation will provide a concise overview of the existing risk assessment framework and its historic applications, as well as a review of more recent efforts regarding chemical prioritization for environmental monitoring and assessment – some of which will be presented by others in this session. Overall, this talk will present an initial state-of-the-science summary of existing prioritization approaches with the future goal of performing an in-depth "meta-analysis" of chemical prioritization approaches for environmental monitoring and assessment.

**645 Modeling Ground Water Contamination by Soil Fumigants in Hawaii and California: Soil Loading and Fate Input are Critical for Validated Predictions** S.Z. Cohen, Environmental & Turf Services, Inc.; G. Pohl, Desert Research Institute; S.W. Wheatcraft, Wheatcraft and Associates; J. Bahme, Bahme & Associates; L. Barnes, A.J. Harding, Environmental & Turf Services, Inc.; Q. Ma, Environmental & Turf Services, Inc. (currently with Exponent) Crop-destroying nematodes have been controlled with soil fumigants, including those with the active ingredient 1,2-dibromo-3-chloropropane (DBCP) and the impurity 1,2,3-trichloropropane (TCP). These products are injected into the root zone of plants prior to planting. Ground water was contaminated with DBCP and TCP following application to pineapple fields on Maui, and TCP was found in ground water in southern California following many years of application to four other crops. Two independent teams modeled the front end – pesticide

application and movement through the root zone – and the saturated zone, without review of monitoring results, in a ‘double blind’ approach. More than 20 yrs each of daily weather records, pesticide application histories, and irrigation practices had to be constructed for the two sites. There was good documentation at the pineapple plantation, although some estimates/interpolation about irrigation and rainfall had to be made. The forensic investigation of the southern California site was more challenging, and it integrated evaluations of historic aerial photographs, farmer interviews, site visits, sparse fumigant sales records, and research of old Extension documents. Soils at both sites were sampled and analyzed to obtain relevant modeling parameters. Site-specific environmental chemistry parameters were derived, focusing on hydrolysis and transport parameters. Root zone contaminant flux was modeled with the EPA’s Pesticide Root Zone Model (PRZM), and HYDRUS-1D was used below the root zone, which fed into saturated zone modeling using MODFLOW-2000 and MT3D. Minimal model calibration was needed to obtain excellent agreement between predicted and at least 10 years of observed concentrations for both locations. This good agreement could only have happened if the decades of front-end loading parameters had been developed correctly. The models were used to predict future contamination.

**646 More Accurate Forensic Investigation of Crude Oil and Coal Tar by Multidimensional GC/MS and GC/MS with Spectral Deconvolution** A. Robbat, Tufts Univ, Chemistry; C. Zeigler, P. Antle, Tufts Univ. Traditional forensic investigations of fossil fuels and their by-products rely on analysis by gas chromatography using either full scan or selected ion monitoring (SIM) mass spectrometry (GC/MS). Full scan GC/MS typically produces unresolved chromatograms, which makes target compound identification difficult due to matrix masking of mass spectral patterns. In contrast, SIM detection, based on one or two ions per target compound, results in dramatic overestimation of analytes and the absence of data to identify other compounds of environmental importance. Polycyclic aromatic hydrocarbons (PAH) and their sulfur analogs (PASH) are used to delineate source, migration pathways, weathering, and threat to human health and the environment. Alkylated PAH and PASH have many common ions, which causes misidentification, errors in quantification, and erroneous weathering diagnostics. Retention indices and mass spectra for more than 200 PAH and PASH were obtained from standards, crude oil, and coal tar by automated sequential GC-GC/MS. The data were used to delineate how soil contaminated with coal tar weathered differently than coal tar protected from the environment. GCxGC/MS provided images that showed which aliphatics and aromatics degraded due to physical vs. biological processes. Measurement precision, accuracy, selectivity and sensitivity will be discussed for chromatograms produced by GCxGC/MS and GC/MS and analyzed by the Ion Signature mass spectrometry deconvolution software.

**647 The Mathematical Estimation of Baseline Concentrations of Metals in Sediments Based on Univariate Distributions and Receptor Modeling** M.J. Bock, T.R. Barber, ENVIRON International Corp.; A.K. Gevertz, ENVIRON International Corp., Zoology. The determination of local baseline concentrations in sediments is often central to the assessment of point sources of contamination. Baseline is typically estimated based on observations taken from a pristine “reference area.” Reference areas are either sampled as part of a site-specific investigation or regional values available from the literature are utilized. These approaches require that a source of baseline observations is available and that the geochemical conditions are similar to the area of interest (e.g., grain size, total organic carbon, source, soil type, bedrock type). In highly industrialized settings, it is often difficult to identify an appropriate baseline dataset. From a geochemical and statistical viewpoint, baseline can be defined as a unique end member with an absence of positive anomalies. This operational definition allows the use of two families of mathematical techniques for the determination of baseline; univariate methods and multivariable receptor modeling. The first class of methods treats contamination as positive anomalies in the univariate distribution of chemical concentrations. Mathematical methods exist for the identification and removal of these positive anomalies. Three statistical methods were identified as having the most promise: (1) the iterative 2-standard deviation technique, (2) the calculated distribution function, and (3) the Gaussian mixture technique. The second class of methods is based on the techniques commonly associated with receptor modeling and source identification in the chemical forensics literature. If the baseline condition is considered as an end member, potential sources of contamination can be

viewed as other end members. Thus, the chemical concentration in any sample is a mixture of baseline and other end members. Statistical techniques such as Polytopic Vector Analysis and Fuzzy C-Means clustering can be used to identify the baseline end member(s). These methods were applied to metals concentrations in surface sediments obtained from publicly available sediment data from the New York/New Jersey Harbor Region impacted by multiple sources of contamination. The results of these analyses show that these methods can be used to identify baseline concentrations of metals in areas impacted by historical sources of contamination.

**648 The Timing of DNAPL Releases in a Complex Fractured Clay Setting** S.A. Flewelling, M. Sharma, Gradient. In this presentation, we will analyze subsurface Dense Non Aqueous Phase Liquid (DNAPL) migration from an industrial facility located in a complex geological setting (fractured clay with discontinuous sand seams). At this site, DNAPL has been found in numerous soil samples and in streambed sediments. Although DNAPL has been found repeatedly, its spatial distribution is discontinuous, resulting in hit-or-miss encounters during sampling events. One of the central questions being evaluated at the site is whether these DNAPL encounters are the result of residual stagnant ganglia, i.e., historical migration, or whether they are from ongoing transport from an upgradient source area, i.e., ongoing migration. Given the complex geological setting and the dual-phase nature of DNAPL and groundwater flow, multiple lines of evidence were utilized to evaluate the timing of DNAPL releases. We will present a framework for evaluating field data within the context of this clay-dominated setting, and then develop a quantitative model that predicts DNAPL travel times through the interconnected fractures and sand seams at the site. This approach can be utilized in other similar settings to address the question of DNAPL migration rates and release timing – key environmental forensics questions in liability assessment and cost allocation disputes.

**649 Tracking Sources of Polychlorinated Biphenyls (PCBs) to Newark Bay Sediments Using Positive Matrix Factorization (PMF)** T. Saba, S. Su, Exponent, Inc. The United States Environmental Protection Agency (USEPA) receptor model, Positive Matrix Factorization (PMF), was used to identify the congener profiles of potential polychlorinated biphenyl (PCB) sources and to estimate their spatial extent of impacts to Newark Bay surface sediments. PCB congener data from different locations throughout the bay were obtained from sediment investigation studies spanning from 2005 to 2008. From PMF simulations, five PCB source profiles were identified as impacting the bay surface sediments. The simulations also pointed to different shoreline areas where PCB inputs are likely to have originated. The congener profile for one of the sources closely resembled a mix of Aroclors 1254 and 1260. This source profile had the highest percent presence in sediment samples collected from the mouths of the Passaic River (north of the bay) and Elizabeth River (south of the bay). The congener profile for another PCB source indicated the prominent presence of congener PCB-15. The spatial concentration distribution of this congener pointed to a shoreline area in the southern portion of the bay. The other PCB source profiles resembled a mix of two or more Aroclors with different degrees of weathering and were also tracked back to source areas along the shoreline. The PCB source profiles obtained from the PMF simulations were corroborated by the loadings obtained from Principal Component Analysis (PCA).

**650 Source Apportionment of Fine Sediment Particles in Highway Stormwater Runoff in the Lake Tahoe Basin** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering, Univ of California, Dept of Civil and Environmental Engineering. Lake Tahoe water column clarity has declined significantly due to urban development and traffic activities. More than 50% of Lake Tahoe’s clarity loss can be attributed to fine sediment particles smaller than 20  $\mu\text{m}$ . To recover the lake water clarity, Lahontan Regional Water Quality Control Board recommended 34% of reduction of the fine sediment particles from urban upland. Stormwater runoff from urban roadways is the largest contributor of fine sediment particles to the lake. One of the critical prerequisites to achieve the fine sediment particle reduction goal is accurate identification of their source profiles and loadings in stormwater runoff. To quantify the loadings of fine sediment particles from major sources such as soil and vegetation debris from adjacent area, traction sand, and pavement wear, source samples and highway snowmelt runoff samples were collected and analyzed for elements and organic markers such as unresolved complex mixture, hopanes, and alkanes. Chemical mass balance model was used to quantify the contributions



of fine sediment particles from eight possible sources. The results indicate surface soil from adjacent side hills was the predominant source, accounting for more than 50% of fine sediment particles in highway stormwater runoff. Molecular markers revealed that almost all solvent extractable organic matter was originated from the wear of asphalt pavement binder, accounted for up to 20%. The contribution of traction sand was less than 5%, which is substantially less than expected. Sampling timing may partially explain this low contribution of traction sand. The results of this study can be used to develop more efficient and cost-effective fine sediment particle load reduction plans and Best Management Practice designs.

**652 World Wide Pb Isotope Distribution in Partial Digestions of Coastal Surface Sediments: Indicator of Leaded Gasoline?** M.M. Larsen, Roskilde Univ, Dept of Science, Systems and Models, National Environmental Research Institute, Aarhus Univ, Marine Ecology; J.S. Blusztajn, Woods Hole Oceanographic Institution. The Galathea 3 expedition circumnavigated the globe in 2006-2007 and collected marine samples from six continents. Twenty three surface sediment samples were analyzed for Pb, Th, U and Pb isotope ratios after microwave digestion with hot concentrated nitric acid, with the hypothesis that the isotope ratio is linked to the geological area and anthropogenic input, mainly from previous use of leaded fuels. Partial digestion was used to enhance the anthropogenic signal, extracting mainly the fine particles and easily soluble part of the sediments. The Pb, Th and U concentrations in the sediments were determined with ICP-Quadrapole MS, and isotope ratios with ICP-multi-collector MS. The measured concentrations were clearly divided into two groups of "background areas" less than 40 mg kg<sup>-1</sup> lead and harbor areas of Sydney (Australia), Charlotte Amalia (St. Thomas, Virgin Islands) and Boston (USA) ranging from 150 to 265 mg kg<sup>-1</sup> lead. The measured isotope ratios was for the most in line with marine upper crust ratios of <sup>206</sup>Pb/<sup>204</sup>Pb vs. <sup>207</sup>Pb/<sup>204</sup>Pb, and in most cases in the range expected for lower crustal xenoliths and marine sediments. Geological distinction between volcanic and mantle signatures was evident except for samples from Accra (Africa) and Greenland, where ratios were beyond the normal ranges. Comparison of the measured Pb isotope ratios of <sup>208</sup>Pb/<sup>207</sup>Pb vs. <sup>206</sup>Pb/<sup>207</sup>Pb with literature distributions of ratios for different sources of Pb in gasoline and coal, and the effect of distance to the harbors of both concentration and Pb isotope ratios will be presented.

**653 Photochemical Degradation of Triclosan: Mechanistic Highlights** S. Kliegman, S.N. Eustis, S. Qian, ETH-Zuerich, Dept of Environmental Sciences/Institute for Biogeochemistry and Pollutant Dynamics; W.A. Arnold, Univ of Minnesota, Dept of Civil Engineering; K. McNeill, ETH-Zuerich, Dept of Environmental Sciences/Institute for Biogeochemistry and Pollutant Dynamics. Triclosan is an antimicrobial compound that is used widely in consumer products, is ubiquitous in the wastewater system, and has been frequently observed in surface waters. Sunlight irradiation of triclosan results in efficient degradation of triclosan as well as formation of small amounts of 2,8-dichlorodibenzo-*p*-dioxin and 2,4-dichlorophenol. Neither the major products, nor a detailed mechanism of these transformations are known, prompting the current study of triclosan photodegradation. The results of experimental and computational studies presented here shed light on the physical and chemical processes that occur when triclosan becomes photochemically excited.

**654 Persistent Organic Pollutants in the North Atlantic Bloom Experiment** L. Zhang, University of Rhode Island, Graduate School of Oceanography; R. Lohmann, Univ of Rhode Island, Graduate School of Oceanography, Univ of Rhode Island. Previous modeling studies have suggested that polychlorinated biphenyls (PCBs) are taken up by planktons and transported downward by sinking particles. These fluxes are dynamically coupled with the air-water exchange processes which may represent a key controlling process in the environmental behaviors of persistent organic pollutants (POPs) locally and globally. A research cruise conducted during the North Atlantic Bloom Experiment (NABE) presented a unique opportunity to verify this hypothesis in the field. From 4/30/2008 to 5/21/2008, air, water, and zooplankton samples were collected every 12 to 24 hrs in a relatively small area south of Iceland on board R/V *Knorr* as the blooms evolved. The partitioning of PCBs and organochlorine pesticides (OCPs) between the dissolved phase and zooplankton (copepod *Calanus*) were determined to assess whether the partitioning is at steady state or kinetically limited, which determines if it can be modeled as a first order loss process. Our field measurement indicated PCBs and small OCPs in the air were fairly abundant at

the beginning of the cruise. The highest air PCB concentrations for seven ICES congeners were found in the Reykjavik at 71 pg m<sup>-3</sup>, which is in line with the findings from passive air sampling in Iceland. As the blooms evolved, although OCPs and small PCBs were still detected in the air, heavy PCBs with more than four chlorines were not found anymore. For example, HCB and PCB-28 were detected in 93% and 68% of all samples with average concentrations of 44 pg m<sup>-3</sup> and 3.6 pg m<sup>-3</sup>, respectively. Whereas PCB-138 were only detected in the samples collected before the blooms evolved. The dissolved PCB concentrations were relatively constant with time, most of which were less than 1 pg L<sup>-1</sup> with a few peak concentrations at several pg L<sup>-1</sup>. An Eulerian two-box model was set up for atmospheric boundary layer and seawater mixed layer including several different source and sink processes of POPs. The modeling simulation results are compared with field measurements, to provide insights into the contribution of partitioning to organic particles, sinking particles export, chemical degradation, and air-water gaseous exchange of PCBs and OCPs to the environmental behavior and fate of these compounds during NABE.

**655 The Fate of Organic Pollutants to Discarded Plastic in Marine Environments** C.M. Rochman, San Diego State Univ and Univ of California, Davis, Marine Ecology/Environmental Health; B. Hentschel, San Diego State Univ and Univ of California, Davis, Dept of Biology; M. Oei, S. Kaye, San Diego State Univ and Univ of California, Davis; E. Hoh, San Diego State Univ, Graduate School of Public Health. Discarded plastic at sea has recently become a problem of great concern in marine environments. Alone they are considered pollution, but it has also become evident that plastics can have an effect on the fate of organic pollutants at sea. In the marine environment, Persistent Organic Pollutants (POPs) such as PCBs, organochlorine pesticides, PAHs, and PBDEs are found adsorbed to discarded plastics at concentrations orders of magnitude above that in the water column. Many marine organisms have been shown to ingest plastic debris directly, and plastics may serve as another pathway for bioaccumulation. The objective of this study is to measure the fate of POPs to marine plastic debris in the ocean. We deployed six types of mass-produced and consumed plastic resin (Polyethylene terephthalate, High-density polyethylene, Polyvinyl chloride, Low-density polyethylene, Polypropylene, Polystyrene) at six different locations in San Diego Bay, CA. These pellets were collected after 1, 3, 6, 9, and 12 months in the Bay and are being analyzed for the rate of adsorption and concentrations of PAHs, PCBs, and organochlorine pesticides. Results to date will be presented at this meeting. At this time we have analyzed data from High-density polyethylene (HDPE) and Polypropylene (PP). We found that after 12 months HDPE adsorbed significantly higher concentrations of PAHs than PP (*p* < 0.001) and PP adsorbed significantly higher concentrations of PCBs than HDPE (*p* = 0.008). We also found differences in the concentrations of plastic-associated POPs among the six sites in San Diego Bay. For example, two of the six sites in the bay concentrate relatively high concentrations of PAHs while another site concentrates relatively high concentrations of PCBs. In general PAHs were 2-3 orders of magnitude higher in concentration than PCBs and organochlorine pesticides. This trend is expected since PAHs are unintentional pollutants of transportation and industrial activity, and PCBs have been banned in the US for decades.

**656 The Measurement of Nonylphenol in Aqueous Aerosols and Marine Waters in the Bermuda Coastal Region and Atlantic Ocean** M. McInnes, Trent Univ, Student, Trent Univ, Chemistry; A.J. Peters, Bermuda Institute of Ocean Science; D.A. Ellis, Trent Univ. Nonylphenol (NP) is an environmentally ubiquitous organic pollutant. Understanding the mechanisms by which it and other pollutants are transported in the environment is of utmost importance. NP exhibits surface active properties and may therefore be subject to transport via aqueous aerosols. Lab studies have shown that aqueous aerosols are subject to increased concentration of organic surfactants when compared with the surface waters they were generated from. This study investigated the concentrations of NP in aqueous aerosols and waters in the Atlantic Ocean and Bermuda coastal region. Aqueous aerosols were collected at heights of 10 m and 20 m from the Research Vessel *Atlantic Explorer* and a land based atmospheric research tower on the coast of Bermuda. NP was also determined in surface waters around Bermuda and to depths of 4000m at the Bermuda Atlantic Time Series (BATS) sampling site in the Atlantic Ocean. Results show that aqueous aerosols produced from the oceans surface are enriched in NP when compared to the surface water they were generated from. This represents a unique route to the atmosphere

and could subject this non volatile compound to long range atmospheric transportation.

**657 Quaternary Ammonium Compounds as Contaminants and Biogeochemical Tracers in Estuarine Waters: A Review** B.J. Brownawell, Stony Brook Univ, School of Marine and Atmospheric Sciences; X. Li, Connecticut Agricultural Experiment Station, Analytical Chemistry; A.C. Doherty, Stony Brook Univ, School of Marine and Atmospheric Sciences, Stony Brook Univ, graduate student; P.C. Fitzgerald, Stony Brook Univ. Quaternary ammonium compounds (QACs) are cationic surfactants with sewage-specific sources to urban waterways. Several are particle-reactive and stable once associated with fine grain coastal sediments. Concentrations of QACs can be remarkably high and are detected with high sensitivity in sediments far from source areas. Several are potent disinfectants leading to questions of effects on microbial communities. However, more toxic and soluble QACs can be highly degraded during sewage treatment. Further fractionation occurs prior to incorporation into sediment beds, after which there appears to be little additional degradation or desorption after extensive transport of resuspended sediments. While very low bioavailability will limit toxicity, the properties of QACs lead to their ability to serve as sensitive tracers of: 1) contaminant source allocation, transport and fate; 2) processes related to the lateral transport and burial of sediments; and 3) microbial source tracking. Recent work shows that concentrations and compositions of QACs are markedly different when suspended matter originates from treated sewage, combined sewage overflows, storm waters, groundwater discharge or resuspended sediments. Illustrative examples will be presented that highlight the potential of QACs as tracers in different applications, and insights into the advantages and difficulties that are inherent in the use of tracers where inputs are not at steady-state over timescales of interest.

**658 Sorption of Aromatic Amines to Soils and Soil Minerals: Implications for the Fate of Emerging Contaminants** D. Vasudevan, T. Arey, Bowdoin College, Dept of Chemistry; M. Newman, T. Zhang, Bowdoin College. Numerous chemicals containing cationic amine functional groups, such as antibiotics, herbicides, and antidepressant drugs, have been detected in surface and ground waters, and some of these pose risks to humans and ecosystems. Understanding and predicting the extent of sorption under a wide range of environmental conditions is key to anticipating potential contamination of groundwater by these chemicals. Cation exchange, the primary mechanism for cationic amine sorption has been well studied. However, there is a lack of knowledge surrounding potential secondary mechanisms of sorption and the soil characteristics that may facilitate secondary, intermolecular interactions between amines sorbed to the surface and adjacent amines in solution. The presence of secondary interaction mechanism can lead to non-linearities in sorption phenomena. If the equilibrium sorption constant ( $K_d$ ), a key parameter in contaminant transport models, is not constant over a wide range of solute concentrations, the sorption is considered non-linear. Transport models that assume a constant  $K_d$  and do not account for non-linearity will under or over predict contaminant transport and provide ineffective assessments. By studying the sorption of a series of model compounds that represent important sub-structures of antibiotics and pesticides, this study addresses three significant gaps in current understanding of cationic amine fate: (i) the influence of compound structural criteria on amine cation sorption, (ii) secondary interactions mechanisms that contribute to non-linearity in sorption phenomena, and (iii) effect of soil properties on sorption linearity and non-linearity.

**659 The Importance of Sorption Kinetics for Modeling Fate and Transport of Contaminated Sediments: Application to the Upper Hudson River PCB Site** P. Israelsson, P. Oates, J. Connolly, C. Forrest, W. Ku, P. Mugunthan, U. Kipka, J. Benaman, R. Petroni, L. Zheng, F. Chen, Anchor QEA, LLC; K. Ziegler, Anchor QEA, Dept of Chemical Engineering, PO Box. A novel framework for simulating the fate and transport of sediment-bound persistent pollutants in surface waters is proposed and applied to the Upper Hudson River (UHR) PCB Superfund Site. Over the past few decades, coupled numerical models of hydrodynamics, sediment transport, and chemical fate and transport have become common in evaluating contaminated sediment site dynamics under various management alternatives. Our work challenges a central assumption typically made within these frameworks, namely the equilibrium partitioning of contaminants between the sorbed and dissolved phases within the water column. We focus on the prediction of downstream cumulative loading of UHR PCBs as it is a

primary regulatory metric. In evaluating the fate of PCBs resuspended either by high flow events or by dredging, we demonstrate that the characteristic settling times of coarser particle size classes (fine sands and larger) are shorter than the timescale for desorption for most of the sorbed PCBs, based on measured UHR PCB desorption rates. Consequently, we argue that equilibrium partitioning in the water column can often be a poor assumption, because it tends to over-predict downstream PCB load by artificially transferring mass into the dissolved phase and away from resuspended coarser particles, thereby reducing the PCB sink associated with the settling of this material. Our solution to this shortcoming of traditional models is a generic multi-rate, size-class-specific kinetic sorption model, i.e., a framework that can represent the kinetics and transport of an arbitrary number of sorbing chemical phases (e.g., labile and resistantly sorbed chemical) independently for each particle size class of the parent sediment transport model. The setting for our work, the UHR PCB Site, is one of the most studied contaminated sediment sites in the world, offering a rich database upon which to build and calibrate a site-specific model application. Of particular note is the availability of data both during baseline conditions as well as during the first phases of the ongoing dredging program, allowing us to investigate and calibrate the influence of sorption kinetics under a range of flow conditions and loadings. The potential inaccuracy caused by equilibrium partitioning can also be characterized. Lastly, we note that although our application concerns UHR PCB dynamics, similar considerations are expected to apply at other sites across a range of conditions and contaminants.

**660 Dissolved Natural Organic Matter – Hydrophobic Organic Contaminant Interactions: Controlling Factors and Environmental Implications** J.V. Kukkonen, J. Akkanen, Univ of Eastern Finland, Joensuu Campus, Dept of Biology. The association with dissolved natural organic matter (DNOM) has been shown to affect the environmental fate and effects of hydrophobic organic contaminants (HOCs). The main findings have indicated that DNOM for example increases the solubility and reduces the bioavailability of HOCs, both processes being very important for the transport of HOCs in the aquatic environment. Since the quality and quantity of DNOM varies greatly both spatially and temporally, site specific estimation and prediction of these effects is difficult. This presentation will challenge some of the current assumptions concerning the DNOM-HOC interactions. First assumption: DNOM quality has major implications for extent of sorption. This is true especially in the case of polycyclic aromatic hydrocarbons (PAHs), but less applicable in the case of polybrominated diphenyl ethers (BDEs). Sorption of the latter appears to be almost independent of DNOM quality. The behavior of polychlorinated biphenyls (PCBs) lies somewhere between that of PAHs and BDEs. Second assumption: sorption of HOC to DNOM is completely irreversible. Recent studies indicate that there is also desorption resistant fraction. The environmental implications of the strongly sorbed fraction are not yet studied. Third assumption: DNOM associated HOCs are not bioavailable. In water phase and non-depletive conditions this holds mostly true. However, there are also indications of increased bioavailability due to low DNOM levels. In addition, recent literature shows that in multiphase systems where solid phase serves as a source for HOCs, DNOM may enhance availability. All these aspects are highly important when considering the effects of DNOM on fate of HOCs in the environment.

**661 Combination of a Stressor-Response Model with a Conditional Probability Analysis Approach to Develop Candidate Criteria from Empirical Data** J. Paul, USEPA, National Health and Environmental Effects Research Laboratory, USEPA, Atlantic Ecology Division; W. munnis, J. Hagy, USEPA. We show that a conditional probability analysis that utilizes a stressor-response model based on a logistic regression provides a useful approach for developing candidate water quality criteria from empirical data. The critical step in this approach is transforming the response data into a binary variable using a threshold that is a desirable management goal for the intended water bodies. A logistic regression analysis on the stressor and binary response data is conducted and the result is interpreted as the probability of missing the management goal. This approach can be viewed as transferring uncertainty in empirical data to an estimate of the probability of missing the management goal. Several examples will be presented to demonstrate the robustness of this approach. The examples involve lakes in the northeast US, wadable streams on the mid-Atlantic US, and estuaries in Florida. This approach is also able to incorporate multiple stressors into the analysis. This capability allows one to look at the interaction among stressors

that can be observed from the data. The issue of nutrient co-limitation in estuaries will be used to demonstrate this capability.

**662 Field vs. Laboratory-based Approaches for Derivation of Aquatic Life Criteria: Just Because We Can, Does It Mean We Should?** R.W. Gensemer, S.P. Canton, GEI Consultants, Inc., Ecological Division. The current practice for derivation of water quality criteria for protection of aquatic life is to use results from standard laboratory toxicity tests with a range of surrogate test species using defined protocols to calculate both acute and chronic criteria. Such criteria have been used for decades to develop discharge permits for a wide range of chemical pollutants under EPA's National Pollutant Discharge and Elimination System (NPDES) and for other uses, such as 303(d) impairment listings and ecological risk assessments. While derivation of these criteria are intended to provide broad levels of protection for aquatic communities exposed to pollutants, uncertainties exist in extrapolating laboratory toxicity test results to protection of natural communities. Recently, however, new approaches have been proposed to derive chemical criteria on the basis of empirical relationships between benthic macroinvertebrate community structure and pollutant concentrations in synoptic surveys. The most recent examples of this approach by EPA include derivation of numeric nutrient criteria in Florida and conductivity benchmarks in central Appalachian streams chemically dominated by sulfate and bicarbonate salts. While use of field data are attractive with regards to environmental realism, identifying causality and minimizing the influence of confounding factors makes derivation of such numeric criteria or benchmarks extremely challenging. Using the conductivity benchmark as a case study, this presentation explores the difficulties of using synoptic field survey data in deriving regulatory aquatic life criteria. Such difficulties include statistical methods used, establishing causality, and approaches for identifying or minimizing the influence of confounding environmental factors. We conclude that, in this case, increases in conductivity (or the ions measured by conductivity) have not yet been shown to be causally related to changes in benthic macroinvertebrate community composition. Furthermore, the confounding factors of benthic habitat characteristics, stream flow, and other anthropogenic influences prevent rigorous application of a numeric aquatic life benchmark based on conductivity.

**663 A Critical Residue Approach Linking Accumulated Zn in Aquatic Insects to Population and Community-level Effects** T.S. Schmidt, US Geological Survey, Water Resources Div, USGS, Water Resources Div; W.H. Clements, Colorado State Univ, Fish, Wildlife and Conservation Biology, Colorado State Univ, Colorado State Univ, Fish, Wildlife and Conservation Biology; R.E. Zuellig, US Geological Survey, Colorado Water Science Center. Whole body Zn concentrations in individuals ( $n = 889$ ) from three aquatic insect taxa (mayflies *Rhithrogena* spp. and *Drunella* spp. and the caddisfly *Arctopsyche grandis*) were used to predict effects on populations and communities ( $n = 149$  samples). Both mayflies accumulated significantly more Zn than the caddisfly. The presence/absence of *Drunella* spp. most reliably distinguished sites with low and high Zn concentrations; however, population densities of mayflies were more sensitive to increases in accumulated Zn. Critical tissue residues (752  $\mu\text{g/g}$  Zn for *Drunella* spp. and 634 for *Rhithrogena* spp.) caused a 20% reduction in maximum (90th quantile) mayfly densities. These critical tissue residues were associated with exposure to 7.4 and 19.2  $\mu\text{g/L}$  dissolved Zn for *Drunella* spp. and *Rhithrogena* spp., respectively. A threshold in a measure of taxonomic completeness (Observed/Expected) was observed at 5.4  $\mu\text{g/L}$  dissolved Zn. Dissolved Zn concentrations associated with critical tissue residues in mayflies were also associated with adverse effects in the aquatic community as a whole. These effects on populations and communities occurred at Zn concentrations below the USEPA hardness-adjusted continuous chronic criterion.

**664 Alternative Methods and Endpoints for Field-derived Exposure-response Modeling: The Case of Stream Invertebrates and Salinity** G.W. Suter, US Environmental Protection Agency, National Center for Environmental Assessment, USEPA, National Center for Environmental A; S.M. Cormier, USEPA, National Center for Environmental Assessment, USEPA, NCEA-Cincinnati; L. Zheng, Tetra Tech, Inc. The time has come to move from research and ad hoc applications of field-derived exposure-response models to developing a consensus of the environmental assessment community about which methods and endpoints are most useful and defensible for standard setting, causal assessment, and risk assessment. That consensus should be based on an understanding of the implications of our choices.

Alternative methods include ordinary linear regression, quantile regression, inflection and break point analysis, and species sensitivity distributions. Endpoints include occurrence or abundance of species, overall species richness and abundance, and multimetric indices. The consequences of choosing among these alternative methods and endpoints will be illustrated with alternatives to the EPA's salinity benchmark for Central Appalachia.

**665 Can the Effects of Insecticide Mixtures be Predicted on Non-target Aquatic Invertebrate Communities?** A.C. Alexander, Univ of New Brunswick, Dept of Biology, Univ of New Brunswick, Dept of Biology and Canadian Rivers Institute; H.M. LeBlanc, Univ of New Brunswick; J.M. Culp, Environment Canada; A.J. Cessna, National Water Research Institute, Environment Canada, Environmental Health National Program, Agriculture and Agri-Food Canada. In this study we pose two questions: (1) Can single-species, single-compound, toxicity test data on non-target aquatic insects (Ephemeroptera, Plecoptera, Trichoptera) predict patterns in stream communities composed of similar taxa that were field-collected, subsampled and exposed to the same compounds individually and jointly? and, (2) Can binary mixtures of two similar Mode-of-Action (MOA) insecticides be treated additively using a Concentration Addition by Toxic Units (TU) approach? To evaluate these questions we field-collected and exposed benthic macroinvertebrate assemblages to four TU doses of either chlorpyrifos or dimethoate individually (control, 0.2, 0.4 and 0.8 TU) and in two, 1:1 mixture doses (0.2 + 0.2 TU and 0.4 + 0.4 TU) in artificial streams. We firstly examined changes in the frequency of different members of the community with respect to actual doses of insecticides using Principle Components Analysis (PCA) and looked for shifts in macroinvertebrate community response. Secondly, we examined whether there were differences in specific taxa or guilds due to treatment with insecticide mixtures compare to control (GLM) and, finally, we compared actual responses to predicted decreases in density using a chi-square approach, where the predicted values were derived from laboratory findings in the EPA Ecotox Database. We found that responses were not uniform with different taxa and guilds responding significantly differently to treatment with either of these two similar insecticides individually or in mixture. We also intend to show when, and suggest why, additive toxicity is suitable for extrapolating from individual responses to population and community level effects for these two organophosphorus insecticides that share the same primary Mode-of-Action.

**666 Causation, Confounding and Field-derived Exposure Response Relationships** S.M. Cormier, USEPA, National Center for Environmental Assessment, USEPA, NCEA-Cincinnati; G.W. Suter, US Environmental Protection Agency, National Center for Environmental Assessment, USEPA, National Center for Environmental A. Field-derived exposure-response relationships have a major advantage: barring errors in measurement, they characterize the real world. Their major disadvantage is that observed relationships may not be causal. This is the opposite of relationships from laboratory tests, which are causal but may relate only to the world of the beaker. Demonstrating real world causation is a difficult problem that has generated controversy from ancient times to the present among philosophers and scientists. We take a pragmatic approach that combines criteria guided inference with an explicit weight-of-evidence process to answer two questions: 1) Does the evidence indicate that the relationship is causal? 2) Does the evidence support any alternative that may account entirely or in part for the apparent causal relationship (i.e., are there confounders)? The criteria used in our approach are six fundamental characteristics of causation: time order, co-occurrence, preceding causation, sufficiency, interaction, and alteration. Evidence is categorized by its relevant causal characteristic and is scored in terms of its strength and quality. The evidence scores are combined to obtain a final score for the putative cause or potential confounder. This approach has the advantage of incorporating all available evidence in a transparent manner.

**667 Field Exposure-Response Relationships Established from (Bio) monitoring Data Provide Insight in SSD-Model Output (Toxic Pressure) Interpretation** L. Posthuma, D. De Zwart, RIVM, Lab. for Ecological Risk Assessment. Strategic research into options for improved analysis of (bio) monitoring data has been triggered by multiple interests, amongst which water quality policies in Europe (the strive to Good Ecological Status) as well as interests in the 'quantitative' ecological meaning of exceedances of Water Quality Criteria. Such exceedances occur frequently, and are a trigger for policy concerns. The current presentation focuses on data collected



in the US as well as Europe, and on the analyses of these data with various diagnostic techniques, with specific attention on locally occurring mixtures of toxic compounds. Over the last decades, emissions of toxic compounds have reduced and water quality has improved, and it can be therefore difficult to establish the relative role of toxic mixtures in causing deviations from Good Ecological Status. It is thereby common that the diagnosis of the role of toxic chemicals is hampered by the issue of "over-parameterisation" of the diagnostic statistics (a.k.a. the "curse of dimensionality"). Often, current analyses yield a 'no-recognized-toxic-signal' answer – be it with little statistical power. That is: such analyses yield no useful answer on the relative role of chemicals, and show neither presence or absence of ecological impacts. In diagnostic analyses of the various geographies, we applied the concept of Species Sensitivity Distributions (SSDs) and mixture modeling to generate acute or chronic toxic pressures per sampling site (PAF or msPAF, multi-substance Potentially Affected Fraction). A chronic PAF of 5% relates to the concept of HC5 (Hazardous Concentration for 5% of the species, equal to the 95% protection level), a well-known concept for deriving water quality criteria. It will be shown that this process was of help in identifying the relative probable role of mixtures in reduction of ecological status in various geographies. Moreover, it will be shown that these analyses provided insights into the meaning of the concept of toxic pressure (msPAF) itself. The presentation addresses not only diagnostics of large (bio)monitoring data sets (as collected e.g., under the Water Framework Directive), but also specifically field Exposure-Response relationships, including new insights in the ecological interpretation of (acute) toxic pressure. The SSD-model appears to help quantifying in a policy-meaningful way the impact magnitude to be expected in contaminated water systems.

**668 Using Microcosm and Field-derived Exposure-Response Relationships to Validate Water Quality Criteria for Metals in Rocky Mountain Streams** W.H. Clements, Colorado State Univ, Fish, Wildlife and Conservation Biology, Colorado State Univ, Colorado State Univ, Fish, Wildlife and Conservation Biology; T.S. Schmidt, US Geological Survey, Water Resources Division. The limitations of single species toxicity tests for predicting ecological effects of contaminants on aquatic communities have been described in the literature. For certain classes of stressors in which direct toxicological effects are unlikely (e.g., nutrients, sediment) or in which standard test organisms are inappropriate (total dissolved solids), field-derived exposure-response relationships have been used to establish standards. The major challenge using field-derived data is to separate effects of the specific stressor of interest from other potentially confounding factors. Our ability to establish exposure-response relationships is greatly improved when a single dominant stressor is present. Using field data from several spatially extensive surveys of over 300 Colorado streams, we established concentration-response relationships between trace metals and several measures of macroinvertebrate community structure. Results showed consistent and predictable alterations in community composition along a gradient of metal contamination. These data were supplemented by a set of 17 stream microcosm experiments that established concentration-response relationships and allowed us to estimate community-level LC20 values for metals. Additional exposure-response data were provided by a long-term (20 year) natural experiment in which we documented macroinvertebrate responses to the removal of heavy metals. Although these data showed significant improvements in water quality and macroinvertebrates over time, communities remained impaired when metal concentrations exceeded the community-level EC20 values. These investigations provided strong evidence that metals associated with historical mining operations were the primary stressors responsible for changes in macroinvertebrate communities. Although exposure-response relationships varied among endpoints, results showed that existing water quality criteria for metals were reasonably protective of benthic macroinvertebrates in Colorado streams.

**669 Translating Tissue Contamination to Sediment Quality Objectives in California Bays and Estuaries** S.M. Bay, Southern California Coastal Water Research Project, Toxicology Dept; B. Greenfield, San Francisco Estuary Institute. The Sediment Quality Objectives (SQOs) adopted by the California State Water Resources Control Board include a narrative objective for protection of human health related to consumption of contaminated seafood. A multiyear effort, informed by stakeholder involvement and scientific review, was used to develop a tiered sediment contamination assessment framework to implement California's human health SQO. The technical approach addresses two assessment questions: 1) do pollutant concentrations

in seafood pose unacceptable health risks to human consumers? and 2) is sediment contamination at a site a significant contributor to the seafood contamination? The first question is evaluated by interpreting seafood tissue concentrations at the site, based on health risk calculations. The second question is evaluated by interpreting site-specific sediment chemistry data using a series of bioaccumulation models based on fish feeding guilds. The models were parameterized and validated for use with chlorinated hydrocarbons in California estuaries and marine embayments. The assessment framework includes three tiers (screening assessment, site assessment, and refined site assessment). Later tiers use a stochastic simulation approach, incorporating information on variability and uncertainty of key parameters, such as seafood contaminant concentrations and seafood consumption rate. Results of application of the framework to data from California embayments illustrate the impact of key regulatory study design parameters on the results, such as site area, species selection, and data quality. The proposed assessment approach would facilitate the consistent application of a risk assessment paradigm in a state sediment regulatory program.

**670 Translating Tissue Levels of Selenium in Aquatic Invertebrates or Fish to Waterborne Concentrations** E. Byron, CH2M Hill, Environmental Services; H. Ohlendorf, CH2M Hill. Throughout the intermountain western United States and Canada the uplift of selenium-enriched marine shales has resulted in a supply of selenium in surface runoff and groundwater that may be evaporatively concentrated or otherwise brought into surface waters at potentially toxic concentrations. Although selenium is an essential micronutrient to animals it may accumulate in diet and tissues to toxic levels in many of these environments. There is regulatory concern for potential selenium toxicity to fish, birds, and hunting or fishing humans coupled with the recognition that selenium exposure and risk occurs mainly through dietary exposure rather than water, directly. As a result, there has been an increasing emphasis in recent years to regulate selenium exposure on the basis of bioaccumulated selenium in the tissue of aquatic invertebrates and fish as well as in secondary consumers, such as in the eggs of water-dependent birds. In this talk we present examples of potentially toxic selenium concentrations in surface water at several western US locations and subsequent attempts to model and translate the measured tissue concentrations back to ambient waterborne concentrations. The modeled relationships may then be used as a tool to regulate waterborne concentrations to levels that would be protective of local fish and wildlife or to help design remediation strategies. The assumptions and causes of variability in these tissue-based models are discussed with regard to the accuracy and precision of translations for selenium between tissues and water.

**671 Spatially-explicit Bioaccumulation Model to Identify Areas of Sediment Associated with Elevated Mercury Uptake by Benthic Fish** J. Conder, ENVIRON International Corporation; R.J. Wenning, ENVIRON International Corporation, Ecology & Sediment Management, ENVIRON International Corporation, Marketplace Tower; F. Achour, F. Colombo, ENVIRON International Corporation. Consideration for active management of local mercury-contaminated sediment in Augusta Bay, Sicily, Italy has been prompted by the detection of mercury in local benthic fish species native to Augusta Bay, despite the fact that concentrations of total mercury in local fish are no different than concentrations observed in other Mediterranean Sea fish. Despite the lack of additional (i.e., beyond ambient) human health and ecological risk posed by local mercury-contaminated sediment via the consumption of local benthic fish, a modeling exercise was conducted to understand the relationship between concentrations in surface sediment (0-20 cm) and benthic fish. Benthic fish collected from the area of the Bay with the highest concentrations of mercury in surface sediment ("Station A"), exhibited slightly higher mean whole body concentrations (0.81 mg/kg, wet weight [ww]) compared to mean concentrations in fish collected from two other sampling areas (0.55-0.61 mg/kg, ww) 2-3 km away. Regression of whole body concentrations of mercury on body weight using data from a species found at all three Augusta Bay locations (red mullet; *Mullus barbatus*) confirmed that this observation was due to increased mercury exposure, not differences in age. Assuming that the benthic fish sampled derive sediment mercury exposure from an approximate 0.1-km<sup>2</sup> home range, Surface Weighted Average Concentrations (SWACs) for total mercury in surface sediment in 0.1-km<sup>2</sup> areas centered over the fish collection locations greater than 60 mg/kg (SWAC for Station A) were equated with the potential for similar or higher mercury exposure in fish than those caught at Station A. A "moving window" geospatial analysis was conducted by estimating the

SWACs for over 1,000 randomly-placed, 0.1-km<sup>2</sup> areas in Augusta Bay. Using the SWACs for each of the moving windows, a contour map showing the 0.1-km<sup>2</sup> SWACs was estimated to identify an area with a SWAC greater than or equal to 60 mg/kg. This analysis explicitly considers the spatial aspects of bioaccumulation of chemicals from sediment by fish, removes bias associated with spatially-unbalanced sediment sampling designs, and can be used to base management actions on both the areal extent and concentration of contaminated sediment.

**672 Delineation of Areas for Sediment Management in the St. Clair River Using Invertebrate Methyl Mercury Tissue Concentrations** L. Richman, Ontario Ministry of Environment; D. Milani, Environment Canada; M. Henning, ENVIRON International Corporation. The St. Clair River was identified as an Area of Concern by the IJC in 1985 because of contaminated fish, degraded benthos, beach closures, and other impairments due to poor water and sediment quality. Currently, sediment management options are under review for three Priority Areas. These areas were identified based on the risk of Hg biomagnification in fish through benthic organisms. Methyl Hg tissue data for oligochaetes collected in 2001/2004 were used to estimate maximum exposure of Hg to fish assuming fish foraged exclusively within the study area. Site specific BMFs were generated using local fish tissue concentrations (pike and redbreasted suckers) and the oligochaete concentrations. These BMFs were divided into a literature derived fish tissue concentration protective of fish (Toxicity Reference Value: 0.2 mg/kg) to obtain a range of target methyl Hg concentrations in invertebrates protective of fish (0.0125 mg/kg – 0.0154 mg/kg). Using anisotropic interpolation, spatially weighted average concentration (SWAC) of methyl Hg in oligochaetes were calculated (0.020 mg/kg). To lower the SWAC below the target concentrations, locations with oligochaete tissue concentrations greater than 0.025-0.027 mg/kg require remediation. To better assess the size of the three Priority Areas requiring remediation, additional benthic tissue data was collected in 2010. Concentrations of methyl Hg in oligochaete tissue, with only a few exceptions, exceeded the target concentrations (range: 0.017 -0.050 mg/kg; median: 0.033 mg/kg). The percent methyl Hg in oligochaete tissue ranged from 4-20% (median 8%). Methyl Hg concentrations in oligochaetes were significantly correlated with methyl Hg and total Hg in sediment ( $r=0.84$ ;  $p<0.002$  and  $r=0.66$ ;  $p<0.04$  respectively). Total Hg in oligochaetes was also significantly correlated with total Hg in sediment ( $r=0.89$ ;  $p<0.0001$ ). Overall, methyl Hg concentrations were consistent in all three Priority Areas with data from 2001/2004. The size of the Priority Areas can be refined using the new data.

**673 Evaluation of One-step Versus Multi-step Partition Modeling Approaches for Relating Selenium Concentrations in Surface Water and Fish** D.K. DeForest, Windward Environmental LLC; K.V. Brix, Univ of Miami, RSMAS, Marine Biology and Fisheries; W.J. Adams, Rio Tinto. For many bioaccumulative chemicals for which trophic transfer is the dominant exposure pathway, the concentration of a chemical in an organism's tissue may be a better indicator of potential toxicity than the concentration of the chemical in the external environment (e.g., surface water). However, in order to develop water quality criteria or guidelines for such chemicals, it is necessary to relate a tissue-based toxicity threshold to a water-based concentration. One example is selenium, an essential element that exists in inorganic and organic forms, with toxicity primarily manifested in aquatic systems via the transfer of organic selenium to oviparous vertebrates (e.g., fish, birds). The bioaccumulation potential of selenium is highly dependent on site-specific biogeochemistry. Mechanistic selenium bioaccumulation models have so far proven to require significant amounts of data and have not been validated for a range of sites. Rather, selenium partitioning modeling approaches based on empirical data have shown more promise. These partitioning models simply rely on coefficients that relate selenium concentrations between two media. In a one-step approach, the selenium concentration in fish or bird tissue (eggs) is related directly to co-located water selenium concentrations. In a multi-step approach, selenium partitioning coefficients are derived for each general step in the food chain: a  $K_d$  to describe selenium partitioning from water to the base of the food chain and trophic transfer factors (TTFs) to describe the transfer of selenium between trophic levels. The selenium  $K_d$  may vary by 1 to 2 orders of magnitude between sites, while TTFs are much less variable between taxa (often varying by a factor of

**674 Derivation of a Tissue-based Selenium Site-specific Objective for the Newport Bay Watershed** C. Claytor, GEI Consultants, Inc.; S.D. Baker, GEI Consultants, Inc., Ecotoxicologist/Wildlife Biologist; S.P. Canton, GEI Consultants, Inc., Ecological Division. Recent efforts have been made by the Santa Ana Regional Water Quality Control Board and Nitrogen and Selenium Management Program to derive a site-specific objective (SSO) for selenium (Se) in the Newport Bay Watershed in southern California in support of a Se TMDL. Using toxicity data and available fish community data, we evaluated their Se fish SSO for the study area and updated the analysis, utilizing relevant Se toxicity data for species expected to be found or which may serve as surrogate species for study area fish. These data were summarized by genus and analyzed tissue type (i.e., whole body or ovary/egg). Based on this analysis of appropriate and relevant data, it appears the bluegill is potentially the most Se-sensitive species in the study area, with a whole-body genus mean chronic value (GMCV) of 7.8 µg/g dry weight. Should a future SSO be based on ovary/egg data, it appears that again the bluegill is most sensitive, with a GMCV of 19.9 µg/g. However, data indicate that bluegill may not be commonly found in the flowing portions of the study area and it is not clear that reproducing populations are present, except in a few atypical, off-channel habitats. Rather, carp and mosquitofish appear to be the most abundant kinds of fish in these areas. In this case, an alternative SSO for those portions of the watershed not supporting reproducing populations of bluegills may be appropriate. We will also review a method for deriving a water column value by back-calculating from a tissue-based SSO, as well as recommendations for more structured site assessments to provide evidence of reproduction (or the lack thereof) through age class structure evaluations, habitat suitability evaluations, a more accurate species list for the watershed, and relative abundance of each species. It is important to note that the protectiveness of the proposed SSO in the context of the overall watershed food web, particularly as it relates to fish-eating birds, has yet to be evaluated and represents a significant uncertainty at this time. A work group comprised of fish and bird experts from private consulting, federal, state, and local agencies is being convened to address this concern.

**676 Tissue Contamination and Seafood Consumption Advisories in Southern California** K. Schiff, Southern California Coastal Water Research Project. Southern California has an active marine fishing community, where 8.4 million fish were landed in 2009. Southern California is also home to some widely contaminated ocean ecosystems including one of the only designated offshore marine superfund sites in the country. This level of contamination can lead to important human health risks for seafood consumers. The goal of this study was to create a southern California regionwide monitoring program for seafood safety. More than 1,700 fish were collected, including the top five-most landed recreational sportfish species. Samples of edible tissues were analyzed for mercury, PCBs and DDTs. Moderate tissue contamination was widespread, with no site exhibiting levels below advisory guidelines for all species, but only a handful of sites exceeding no consumption advisory thresholds. Mercury and PCBs were the most widespread contaminants. Despite the DDT superfund site, DDT tissue concentrations were uniformly below advisory guidelines. Embayments had greater mercury and PCB concentrations, and exceeded advisory guidelines more frequently than open coastal sites. The greater embayment concentrations compounds health risk since angler surveys illustrate these habitats are where the greatest frequency of fishing occurs.

**677 A Novel QSAR Modeling Strategy: Variable Selection Method Based on Variable Interaction via Uniform Design Cross-Validation** Z. Yi, Nanjing Univ, Chinese Academy of Sciences, Research Center for Eco-environmental Sciences; A. Zhang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, State Key Laboratory of Environmental Chemistry and Ecotoxicology; Y. Mu, J. Fu, Y. Sun, Chinese Academy of Sciences, Research Center for Eco-environmental Sciences. With the development of structural characterization techniques, variable selection method plays a key role in screening molecular descriptors to establish predictive and robust quantitative structure-activity relationship (QSAR) models. Random researching methods such as genetic algorithm and art colony algorithm may not ensure that the same global optimal subset is found, while it is impossible to use all-subsets regression for subset selection when there is a large variable candidate database. In order to tackle the problem, a novel QSAR modeling strategy, variable selection method based on variable interaction via uniform design cross-validation (VSMVI-UDCV), was proposed in the present study. Specifically, variable selection

method based on variable interaction (VSMVI) was developed on the basis of three techniques, forward regression, variable selection method based on prediction (VSMPI), and group methods of data handling. The new method can solve the subset screening problem much faster than VSMP, exhibiting obvious advantages over VSMP when handling large set of variables. In addition, the characteristics of uniform design were adopted to modify leave-multiple-out cross-validation (LMOCV) and establish uniform design cross-validation (UDCV) to get the most possible amount of information with the minimum number of model validation. By using uniform design table, the validation strategy is achieved by setting sample number as a factor level and different factor as different LMOCV splitting way. The new strategy differs from the other variable selection procedures related to all-subsets regression by the two major features: (1) The search of various optimal subset search is controlled by the statistic  $q^2_{UDCV}$  (the correlation coefficient obtained using UDCV technique) rather than the correlation coefficient obtained in the modeling step ( $r^2$ ) or  $q^2_{LOO}$  in the leave-one-out cross-validation step. (2) The searching speed of all optimal subsets is expedited by the statistic  $r_{int}$  (the interrelation coefficient between the pairs of the independent variables), together with  $q^2_{UDCV}$ . Finally, the performance of VSMVI in variable selection was assessed and verified by Selwood dataset, while the validity of UDCV was evaluated by comparison with Monte Carlo cross-validation method.

#### 678 A QSAR-based Two-stage Prediction for Estimating Mixture

**Toxicity** J. Kim, KIST Europe, Knowledge Research Group, Univ of Koblenz-Landau, Institute of Environmental Sciences; S. Kim, KIST Europe, Chemical Management Lab.; G.E. Schaumann, Univ of Koblenz-Landau, Institute of Environmental Sciences. Chemical risk assessment frequently focuses on single chemical substances, although most living organisms are actually exposed to chemical mixtures rather than individual substances. Much evidence on mixture toxicity shows that individual substances at levels below no-observed-effect concentrations (NOECs) may elicit toxicity due to the combined effects among substances. Hence, mixtures risk assessment covering the combined effects needs to be considered for human health and the environments. Conventionally, concentration addition (CA) and independent action (IA) models based on the concept of additive toxicity are often used to estimate the mixture toxicity of similarly- and dissimilarly acting chemicals, respectively. However, organisms and the environments are simultaneously exposed to both types of chemicals. Therefore, it practically needs to develop an integrated model to predict mixture toxicity from various chemicals. Two-Stage Prediction (TSP) model has been developed as an integrated addition model to carry out the CA and IA calculations stage by stage. But the use of the conventional TSP is limited if knowledge on the modes of toxic action on every mixture component is not readily available. The objective of this study is to develop a QSAR-based TSP model for estimating mixture toxicity in the environment risk assessment. For this purpose, the categorisation of mixture constituents using computerised analysis of chemical similarity is applied as a part of the prediction of mixture toxicity. The results show the QSAR-based TSP can overcome the limitation of the conventional TSP model, as well as the categorisation of mixture constituents based on the chemical structure can be applied to predict mixture toxicity effectively.

#### 679 Adaptive Responses in Female Fathead Minnows Exposed to an Aromatase Inhibitor: Computational Modeling of the Hypothalamic-Pituitary-Gonadal Axis

M. Breen, North Carolina State Univ, Biomathematics Graduate Program; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; D. Bencic, USEPA, Office of Research and Development, USEPA; M.S. Breen, USEPA, National Exposure Research Laboratory; K.H. Watanabe, Oregon Health & Science Univ, Dept of Environmental and Biomolecular Systems; A.L. Lloyd, North Carolina State Univ, Biomathematics Graduate Program; R.B. Conolly, USEPA, Integrated Systems Toxicology Division. Exposure to endocrine disrupting chemicals can affect reproduction and development in both humans and wildlife. We are developing a mechanistic computational model of the hypothalamic-pituitary-gonadal (HPG) axis in female fathead minnows to predict dose-response and time-course (DRTC) behaviors for endocrine effects of the aromatase inhibitor, fadrozole. The model includes two feedback regulatory loops within the HPG axis that mediate adaptive responses to endocrine stress. One

regulatory loop controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the brain, and the other regulates LH and FSH receptor recycling in the ovary. Data on plasma E2 and ovarian CYP19A mRNA from two experiments with a post-exposure recovery phase were used to develop and evaluate the model. In the experiments, fathead minnows were exposed to fadrozole at 0, 3, or 30  $\mu\text{g/L}$  for 8 days followed by a 8-day recovery phase (experiment 1) or to fadrozole at 0, 0.5, or 30  $\mu\text{g/L}$  for 8 days followed by a 20-day recovery phase (experiment 2). Adaptive changes in plasma E2 levels occurred during exposure and overshoot occurred post-exposure. Initial efforts to identify parameter values providing good fits to the plasma E2 data were only partially successful, suggesting the possibility that additional regulatory loops in the HPG axis are needed in the model. Ongoing efforts are evaluating both this possibility and new approaches to parameter estimation for the current model structure. This study illustrates the value of computational modeling for (1) examining the possible dynamic behaviors of a given model structure and (2) exploration of modifications to model structure leading to novel hypotheses regarding the regulatory biology associated with the observed adaptive responses. This abstract does not necessarily reflect US Environmental Protection Agency policy.

#### 680 Application of a Genome Wide Live Cell Array System in Ecotoxicity Assessment

X. Zhang, Toxicology centre, Univ of Saskatchewan, State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing Univ, School of the Environment; G. Su, Nanjing Univ, School of Environment; X. Hu, Nanjing Univ; J. Giesy, Univ of Saskatchewan; H. Yu, Nanjing Univ. Continuous real time monitoring of genome-wide responses at cellular level enables new understanding of the complex biological process modulated by exposure to environmental chemicals. We have applied a high throughput live cell reporter assay system in chemical toxicity assessment. This live cell reporter assay system contains 1820 *Escherichia coli* K12 promoter-GFP reporter strains. Over 30 different single test chemicals and mixtures have been tested to assess their cytotoxicity and genomic responses. The powerful utilities of this system have been explored in the following areas: 1) chemical classification; 2) prediction on ecotoxicity; 3) mechanistic understanding; 4) mixture toxicity prediction.

#### 681 Bayesian Process Analysis of Time-Course Microarray Expression Data to Elucidate Molecular Mechanisms for Chemical-Induced Reversible Neurotoxicity

P. Gong, SpecPro Inc., Environmental Services. Both carbaryl (a carbamate insecticide) and cyclotrimethylenetrinitramine or RDX (an explosives compound) can cause reversible neurotoxicity in the earthworm *Eisenia fetida*. The goal of this study was to better understand its underlying mechanism using a systems biology approach. We generated a time-series gene expression dataset that captured 27 snapshots of 44K unique transcripts in the course of 6-d exposure (13 time points) and 7-d recovery (14 time points) in earthworms received carbaryl (20  $\text{ng}/\text{cm}^2$ ), RDX (2  $\mu\text{g}/\text{cm}^2$ ) or neither (solvent control), in addition to 4 snapshots during acclimation. A Bayesian Learning and Optimization Model (BLOM) was developed to infer gene interactions. We analyzed BLOM-inferred gene networks for three treatments and two experimental phases using different gene sets. Our results show that (1) physiological recovery measured by MGF conduction velocity was reflected in both the number and the restored expression level of altered genes; (2) physiological response also correlated with the number and connectivity strength of disrupted gene interactions; and (3) RDX and carbaryl affected different gene interactions in various KEGG pathways. Furthermore, our data support that carbaryl inhibits acetylcholinesterase while RDX binds to GABA<sub>A</sub> receptor. Networks comparison also reveals that RDX induced interactions between genes coding for GABA<sub>A</sub> receptor and that for Dlc1/2 (dynein light chain-1 and -2). These interactions disappeared during the recovery phase. Although the majority of altered gene interactions and gene expressions were restored during the recovery, some important ones involved in pathways such as neurodegenerative diseases remained aberrant, implying potential long-term adverse impacts of RDX and carbaryl.

**682 ICE: Computational Toxicology Gone Wrong?** P. Craig, G. Hickey, Durham Univ, Mathematical Sciences. The USEPA interspecies correlation estimation (ICE) programme is a suite of log-log linear models fitted from existing ecotoxicity databases, with the purpose of predicting the unmeasured toxicity to some species of a chemical from measurements for other so-called surrogate species. An intended use of ICE is to reduce the number



of species tested in order to fit a species sensitivity distribution (SSD) by simultaneously predicting toxicity to a large number of species from measurements of toxicity to only a few, possibly just  $n=1$ . This semi-in silico method is motivated by restrictions on the minimum sample size required for SSD based hazard assessment; for example, REACH requirements vary from 10 to 15 species. We show that the concept is statistically indefensible on three grounds. Firstly, we examine whether the underlying ICE models themselves are informative about sensitivity of individual species or, when applied, are just making noisy predictions of central tendency. Secondly, we examine whether ICE-SSDs are conceptually coherent, show how one might exploit a suite of ICE models, and quantify, by means of a residual analysis, how well ICE-SSDs predict hazardous concentrations. Thirdly, we demonstrate that the implementation of ICE in USEPA Web-ICE software, and in particular quantification of uncertainty which is necessary for adoption under REACH, is erroneous and in some cases nonsensical. We conclude that, as it stands, ICE is a tool not fit for regulatory risk assessment of chemicals and indicate more suitable models, based on Bayesian hierarchical modelling, which maximize the value of information in existing ecotoxicity databases whilst being statistically defensible. The experience with ICE shows that the advent of powerful computing is not sufficient on its own to be useful for computational toxicity. One must also use the right statistical tools in the right ways.

**683 Prediction of the Aquatic Toxicity of Triazoles and Benzotriazoles by QSAR** E. Papa, Univ of Insubria, QSAR Res. Unit Environ. Chem/Dep. Structural Functional Biology, Univ of Insubria, DBSF; S. Kovarich, P. Roy, S. Cassani, E. D'Onofrio, Univ of Insubria; P. Gramatica, Univ of Insubria, QSAR Res. Unit Environ. Chem. Ecotox./Dep. Structural & Functional Biology. Triazoles and benzo triazoles (TAZ/BTAZs) are synthetic molecules already included in the list of substances of very high concern (SVHC) in the European regulation of chemicals REACH. These chemicals are widely used to obtain pharmaceuticals and agricultural products, and have a wide application as anti-corrosives, cleaning agents for textiles, flame retardants, photographic emulsions, as well as they are components of liquid deicing agents for aircraft and airport runways. Experimental evidence show that TAZ/BTAZs are distributed throughout the environment and in particular in the water compartment. Despite their known toxic activity on different organisms and non-target species, and in particular on the aquatic environment, only a limited amount of data is available for a comprehensive characterization of the environmental and toxicological profile of TAZs and BTAZs. Therefore, in order to increase the level of knowledge necessary to define the actual environmental impact of these potentially hazardous emerging contaminants, they have been included among the four classes of chemicals studied in the European FP7 Project CADASTER. This study focusses on the development of multiple linear regression (OLS) QSAR models for the acute toxicity of TAZ/BTAZs on three key biological levels of an aquatic ecosystem (Alga, *Daphnia* and Fish). Moreover, Quantitative interspecies correlations were determined to extrapolate *Daphnia* toxicity to Fish. The best models were validated for their internal robustness and external predictivity. The reliability of predictions was always checked by the leverage approach in order to verify the structural applicability domain of the models. Finally, a PCA analysis of the experimental toxicity data reported for the studied species was used to rank the studied TAZ/BTAZs according to their toxicity profile in the considered aquatic scenario.

**684 Quantum Chemical Prediction Models on the Environmental Photochemical Behavior of Organic Pollutants** J. Chen, Dalian Univ of Technology, School of Environmental Science and Technology, Dalian Univ of Technology, Professor; S. Zhang, Q. Xie, J. Zhou, Dalian Univ of Technology. Computational prediction methods are crucial for ecological risk assessment of environmental chemicals and newly synthesized chemicals prior to commercialization. Photochemical degradation is an important transformation pathway of persistent toxic chemicals in surface waters, in atmosphere, and on plant/soil surfaces. It is impossible to experimentally evaluate the photochemical behavior for all the (potential) environmental chemicals. Thus, it is necessary to develop computational models on environmental behavior of environmental chemicals. Our studies show that it is feasible to develop predictive models on environmental photochemical behavior of chemicals. The results include: (1) Predictive models on direct photolysis quantum yields, rate constants as well as bimolecular reaction rate constants with reactive oxygen species (ROS) for aromatic compounds were developed using quantum chemical parameters [1-3]. (2) A density

functional theory (DFT) based prediction model on photolytic kinetics of hydroxyl radicals ( $\bullet\text{OH}$ ) induced photooxidation was developed for semi-volatile organic pollutants in atmosphere [4]. (3) DFT based models were developed to predict and explain the different photodegradation kinetics between molecular and ionized hydroxylated polybrominated diphenyl ethers (HO-PBDEs). (4) DFT computational methods were developed to predict the photochemical transformation pathways by evaluating the energy/electron transfers between environmental chemicals and oxygen molecules/water constituents [5]. References (1) Jingwen Chen, W. J. G. M. Peijnenburg, Xie Quan, et al. Chemosphere, 2000, 40(12):1319-1326. (2) Jingwen Chen, W. J. G. M. Peijnenburg, Xie Quan, et al. Environ. Pollut., 2001, 114(1):137-143. (3) Ya'nan Wang, Jingwen Chen, Xuehua Li, et al. Atmos. Environ., 2009, 43(5):1131-1135. (4) Jing Zhou, Jingwen Chen, Chi-Hsiu Liang, et al. Environ. Sci. Technol., dx.doi.org/10.1021/es200087w (5) Siyu Zhang, Jingwen Chen, Xianliang Qiao, et al. Environ. Sci. & Technol., 2010, 44(19):7484-7490.

**685 Reducing Risk by Reducing Hazard: Use of Chemical Hazard Screening as the First Step in the Assessment Process** C. Robertson, Hewlett-Packard; P. Mazurkiewicz, H. Holder, HP. The Green Screen for Safer Chemicals (Green Screen) tool is a comparative chemical hazard assessment tool that is used to identify safer chemical alternatives in the design process. Based on the principles of green chemistry that assert that the best way to reduce risk is to reduce hazard, the Green Screen provides a simple 1-4 benchmark score that can help identify less hazardous chemical and non-chemical alternatives. A business case for screening alternatives using the Green Screen can be made based on the cost savings realized by avoiding multiple substitutions. Substituting materials in a complex supply chain can be costly, and if the replacement chemicals face future regulation, businesses may incur that cost multiple times. Selecting inherently less hazardous alternatives as the first step in the assessment process reduces the risk that the substitutes will face future restrictions. This presentation will discuss the incorporation of hazard based screening in the selection of materials for PVC-free power cords at Hewlett-Packard. Because the transition from PVC to PVC-free materials is voluntary, the importance of ensuring that the non-PVC alternatives are truly better than PVC materials is paramount. Use of the Green Screen has led to selection of materials that are inherently less hazardous, resulting in finished products that have a lower impact on the environment and human health.

**686 Improving Environmental Sustainability in the Marketplace One Step at a Time** C. Kelly, AMEC Geomatrix, Inc. Successful green chemistry requires an efficient screening of a chemical's intrinsic toxicity within the context of the final product the chemical will exist in. Providing numerical rankings to our client's material choices is an integral part of the decision-making process so the client's R&D Dept can develop ways to avoid, replace, or eliminate the more toxic products. However, the potential exposure routes and typical use in the marketplace cannot be separated from the process. We will present the scoring system and decision tree developed with a client in the sportswear industry and highlight the limitations of its application in addition to the tools that have proven useful to make it robust for a variety of chemicals. We will provide example chemicals that ideally fit an automated scoring system and others that may lead to misleading scores. We will present how we avoid inadvertently including chemicals that should be removed from the product stream and inadvertently excluding other potential "green" chemicals.

**687 Green Disinfectants – A Life Cycle-based Framework to Address the Hazard, Exposure, and Risk of These Products** C. Baldwin, S. Cooperstein, Green Seal. Cleaning programs with disinfectants are frequently required in institutional settings including health-care facilities, child care centers, schools, hospitals, long-term care facilities, and others. Many active ingredients in disinfectants are potentially hazardous to human health and the environment. They may contain chemicals that cause cancer, reproductive disorders, respiratory ailments (including occupational asthma), and other human health effects. In addition, some of these products contain persistent bioaccumulative and toxic chemicals (PBTs), are classified as hazardous waste, and/or otherwise contribute to environmental pollution during their manufacture, transport, use, and/or disposal. Furthermore, resistance of bacterial pathogens to antibiotics has increased worldwide, leading to treatment failures in human and animal infectious diseases. As a result, effective cleaning programs minimize use of disinfectants through

targeted treatment plans. Such a targeted plan involves the identification of which surfaces require high level disinfection (high-touch surfaces with a high risk for disease transmission), those that require low-level disinfection, and those surfaces that pose no infection risk, thereby minimizing the unnecessary use of toxic, high-level disinfectants. However, to further reduce the hazards from disinfection, less hazardous products should be used for the areas identified as needing this treatment. Currently, all disinfectants are classified as antimicrobial pesticides and registered under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Through the implementation of this law, these products are prohibited from bearing comparative claims, including "green" claims. However, there are greener disinfectants. This presentation will discuss the attributes of preferable disinfectants and how this fits into a life-cycle based evaluation program, namely the Green Seal ecolabel program.

**688 Green Chemistry Strategies: A Synthetic Chemists Perspective** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences. The talk will include introducing green chemistry metrics used by research and development chemists in the design of 'safer' synthetic methods. Recent examples from our group in Dublin will showcase, how the 12 Principles of Green Chemistry can be applied. The talk will include atom economy, E factor, toxicity and biodegradation data for solvents, catalysts, and reagents. Consideration of the sustainability of raw materials, with the environmental cost due to the preparation of starting materials or reagents is also an important factor often not included. In our presentation we will give an overview of the importance of all these factors, and their careful consideration in the design of a green synthesis.

**689 Evaluating Overall Degradability of Chemicals with QSARs** T.N. Brown, Helmholtz Centre for Environmental Research UFZ, Dept of Analytical Environmental Chemistry; J.A. Arnot, F. Wania, Univ of Toronto Scarborough, Dept of Chemistry, Dept of Physical and Environmental Sciences; K. Goss, Helmholtz Centre for Environmental Research UFZ, Dept of Analytical Environmental Chemistry. Predictive methods are required to develop and design green and sustainable chemistry to reduce potential risks as a result of chemical exposure. Overall degradability of a chemical in the environment is a key determinant of chemical exposure, and thus potential risk. Overall degradability in the environment is a function of chemical partitioning in the physical compartments of the environment (i.e., multimedia distribution in air, water, soil and sediment) and the rates of degradation in the physical compartments of the environment. Depending on the chemical usage, mode of emission to the environment, and qualities of the receiving environment, a chemical may have a different environmental fate and therefore different overall degradability. Using a combination of multimedia mass balance exposure models and quantitative structure activity relationships (QSARs) a set of structural features are identified that have a high susceptibility to degradation regardless of their mode of emission. QSARs are developed for a number of different abiotic and biotic transformation processes using Iterative Fragment Selection (IFS). IFS is an integrated method of generating QSARs that includes dataset splitting, descriptor generation and model selection which requires no a priori knowledge (e.g., expert judgement) for the selection of chemical descriptors (fragments). A number of structural features in the form of fragments are identified that cause a contaminant to have a high potential for degradability from multiple degradation pathways.

**690 Incorporating Risk(s) in Environmental Ranking and Scoring of Chemical Ingredients** S. Thakali, K.R. Reid, T. Verslycke, M. Sharma, Gradient. Numerous ranking and scoring approaches for evaluating and assessing chemicals and products have been developed to help identify 'greener' alternatives. These approaches have been modified and improved such that they are now increasingly applied to chemical ingredients in consumer products. Some of these tools evaluate chemicals via qualitative, hazard-based criteria, while others are used for ranking and priority-setting; still others seek to "label" consumer products based on human health and ecological risks. However, many of these scoring and ranking frameworks generally do not explicitly consider potential risk(s) because they fail to integrate both hazard and exposure from typical product use. We have reviewed several available ranking and scoring tools and developed a risk-based approach to evaluate the relative environmental safety of surfactants widely used in personal care products (PCPs), in household laundry products, in

domestic and industrial cleaners, and in a variety of industrial applications. In our application of various approaches for scoring and ranking these widely used surfactants, we used readily-available environmental fate/exposure and hazards data. We will present a review of several chemical/product ranking and scoring frameworks, and compare and contrast the results of the applications of various approaches—including our proposed risk-based approach—to score and rank environmental safety of these widely used surfactants.

**691 Green Organocatalysis** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences; T. Hayes, Dublin City Univ, School of Chemical Sciences and National Institute for Cellular Biotechnology. Enantiopure compounds are of great importance to the chemical industry especially as pharmaceuticals. One strategy in synthesizing enantiopure compounds involves the use of asymmetric catalysts such as organocatalysts. These catalysts are small chiral organic molecules used in substoichiometric amounts to promote organic reactions. However, for organocatalysts to be classified as green reagents, their toxicity and biodegradation needs to be assessed. For instance, polycyclic aromatic hydrocarbons, known pollutants, are to be found in the structure of chiral phosphoric acids and may be a cause for concern. Our efforts are to determine which chiral BINOLs or proline derived organocatalysts are biodegradable and exhibit low toxicity in microbial assays. Herein, we present results from our anti-microbial study of TADDOLs, BINOLs and proline derived organocatalysts. Minimum inhibitory concentration values (MIC) are determined using the microtiter broth dilution assay. The synthesis of a series of polysubstituted BINOLs is also reported.

**692 Toxicity of Green Household Product Formulations to Larvae of the Grass Shrimp, *Palaemonetes pugio*** J. Miller, E. Dillihay, The Citadel; W. Boyd, Benedict College; J. Weinstein, The Citadel, Dept of Biology. Although it is generally assumed that green products contain individual ingredients that are less toxic to both humans and the environment, little research has been conducted to examine whether or not the overall end product formulations are less toxic to aquatic organisms. This study examined the toxicity of several household green products relative to their conventional counterparts. Using the larvae of the daggerblade grass shrimp (*Palaemonetes pugio*), the toxicity of seven green product formulations (Seventh Generation Dishwashing Gel, Green Works All-purpose Cleaner, Green Works Dish Detergent, Earth Options Insect Killer, Tom's Mouthwash, Martha Stewart Bathroom Cleaner, and Seventh Generation Laundry Detergent) were determined and compared to that of categorically equivalent conventional product formulations. All of the products were assessed using 48-hour toxicity tests; the results of these tests were statistically analyzed in order to determine median lethal concentrations (LC50s), and then compared to one another using an LC50 ratio test. Of the seven categories of household products tested, in only two cases was the green product formulation less toxic than both tested conventional product formulations. In four cases, the green product was equally toxic compared to at least one tested conventional product. And, in one case, the tested green product was more toxic than both tested conventional products. Research examining the relative toxicity of these same product formulations following biodegradation will also be discussed. This research demonstrates that green consumer product formulations are not necessarily less toxic than their conventional counterparts. These results also suggest that the toxicity of end product formulations should be considered in the overall green product evaluation framework.

**693 Effects of the Massachusetts Bay Sewage Outfall on Soft-bottom Benthos Assessed Using Canonical Ordination and an Evaluation of Pollutant Indices** E. Gallagher, A. Evgenidou, Univ Of Massachusetts Boston, Environmental, Earth & Ocean Sciences; K. Keay, Massachusetts Water Resources Authority; J. Blake, N. Maciolek, AECOM; D. Dahlen, Battelle New England. We assessed the effects of the MA Bay sewage effluent outfall on MA Bay infaunal benthos using canonical ordination of chord-normalized expected species shared (CNESS) faunal dissimilarities. This ordination, called CPCA-H, is based on transforming species abundances to hypergeometric probabilities and offers an ecologically meaningful alternative to CANOCO's ordination of chi-square distances. Triplots indicate that depth, total organic carbon and % fines are the major determinants of benthic community structure and that the outfall had little effect on benthic community structure. The outfall's discharge increased the concentrations of

*Clostridium perfringens* spores, a sewage tracer, in depositional areas near the outfall, and our CPCA-H found a positive association of these spores and the abundances of three polychaete species: *Mediomastus californiensis*, *Tharyx acutus*, and *Aricidea catherinae*. Our MA Bay canonical ordination allows us to evaluate several widely used pollution indices based on the pollution sensitivity of infaunal species. Canonical ordination and inverse analysis using ter Braak & Juggins' C2 program allow us to determine the association between infaunal species abundance and pollutant gradients and to assign pollutant scores and tolerances for individual species. As a related measure of pollutant impacts using hypergeometric probabilities, we've found Fisher's alpha and Hubbell's theta provide strong indicators of long-term changes in community structure and departures from the expected evenness based on these species abundance distributions can be an effective tool for identifying impacted benthic communities using Sanders-Hurlbert rarefaction.

**694 Effects of Sewage-impacted Sediments on Growth and Reproduction in the Benthic Crustacean *Leptochierus plumulosus*** A. McElroy, Stony Brook Univ, School of Marine and Atmospheric Sciences; A. Zulkosky; L. Ferguson, Duke Univ, Civil and Environmental Engineering; B. Brownawell, Stony Brook Univ, School of Marine and Atmospheric Sciences. Sediments serve as a major source and sink for hydrophobic chemicals in aquatic ecosystems, and in urban areas as a repository for a suite of sewage derived contaminants. This study was undertaken to assess the toxicity of sediments from two heavily sewage impacted urban sites, Boston Harbor, MA and Jamaica Bay, NY. As the detergent breakdown product nonylphenol (NP) and its ethoxylated precursors (NPEOs) have been found to be present at extremely high concentrations in Jamaica Bay, we measured levels of these contaminants as well as another sewage marker, *Clostridium perfringens* spores in sediments from both sites, and also compared the toxicity of sediments from these sites to those from a reference site dosed with NP. Growth, reproduction, and survival endpoints in the benthic amphipod, *Leptochierus plumulosus*, exposed to field collected or NP dosed sediments were used for toxicity assessment. Sediments from Jamaica Bay had higher levels of NP and NPEOs than sediments from Boston Harbor, but had more similar levels of *C. perfringens* spores. Boston Harbor sediments were not significantly toxic in any endpoint measured (10-day adult mortality, or 28-day reproductive output or average growth), while one of the Jamaica Bay sites, Bergin Basin, was acutely toxic in 10 day adult tests, and the other, Grassy Bay, led to significant reductions in reproductive output after 28 days. Experiments with NP dosed sediments determined the 10-day LC50 to be 150 µg/gdw with no reproductive or growth effects quantified as sediment concentrations as high as 60 µg/gdw. Sediments examined in this study were collected in 2000, almost a year after the sewage outfall in Boston Harbor was relocated to Massachusetts Bay. The reduced levels of NP and NPEOs and sediment toxicity observed, indicate levels and impacts of sewage-derived contaminants may already have been decreasing at that time, and demonstrate sediment toxicity in Boston Harbor at that time was less than that observed in another urban estuary with continuing sewage inputs, Jamaica Bay. This study was supported by the Massachusetts Environmental Trust.

**695 The Relationship Between Improved Sediment Quality and Indicators of Flounder Health in Boston Harbor** C.D. Hunt, Battelle; M. Moore, Woods Hole Oceanographic Institute; D. Dahlen, Battelle; M. Hall, Massachusetts Water Resource Authority. Loading of contaminants to Boston Harbor has been reduced through harbor cleanup initiatives implemented over the past thirty years. The 1972 Clean Water Act and other factors such as the recession in the 1980's reduced the effects from inefficient industrial treatment and discharge practices. The Massachusetts Water Resources Authority Harbor Cleanup project with its excellent and aggressive source reduction program, removal of sewage sludge discharge, commissioning of primary and secondary sewage treatment, and offshore discharge continued the improvements through the 1990's and early twenty-first century. These reductions have demonstrably improved the quality of surface sediments in the harbor. The improved quality of Boston Harbor is also evident in the recovery of the health of a major living resources (e.g., winter flounder) of the Harbor and a general reduction in contaminant levels in fish and shellfish resources in the system. Moreover, the high prevalence of liver neoplasm (tumor) in winter flounder (*Pseudopleuronectes americanus*) collected from Boston Harbor in since 1984 show a steady decrease as do concentrations of organic contaminants. The prevalence of Centrotubular Hydropic Vacuolation (CHV), a precursor to liver tumors has also declined

in the harbor and is presently half of what they were during the early 1990s. This paper explores the relationship among the improved sediment quality and the decreasing CHV trend.

**696 Estimating Narcosis Potential in Boston Harbor Sediments Using Passive Samplers and Two-dimensional Gas Chromatography** A. Tcaciuc, Massachusetts Institute of Technology, Civil and Environmental Engineering; J.K. MacFarlane, Massachusetts Institute of Technology, Dept of Civil and Environmental Engineering; M. Nipper, Texas A&M Univ-Corpus Christi, Center for Coastal Studies; R.K. Nelson, Woods Hole Oceanographic Institution, Dept of Marine Chemistry and Geochemistry; R. Carr, USGS CERC, Marine Ecotoxicology Research Station; J. Biedenbach, USGS/BRD, Marine Ecotoxicology Research Station; C.M. Reddy, Woods Hole Oceanographic Institution; P.M. Gschwend, Massachusetts Institute of Technology, Dept of Civil and Environmental Engineering. Sediments from around Boston Harbor always exhibit contamination with polycyclic aromatic hydrocarbons (PAHs) and many other hydrophobic organic chemicals (HOCs) as a result of commercial shipping, urbanization, and/or industrial activity. While individual contaminants may exhibit specific toxic modes of action, all HOCs contribute to toxicity through a common baseline narcosis mechanism. HOCs are usually present in the environment as complex mixtures; but toxicity assessments so far, tend to focus on a certain subset of identifiable chemicals with known toxic effect concentrations. Here we propose a method to determine the integrated narcosis toxicity due to all the HOCs present in contaminated sediments. For that we make use of the fact that narcosis is nonspecific, additive, and thought to occur beyond critical body burdens of approximately 0.01 g HOCs/g of lipid. By using polyethylene samplers and two dimensional gas chromatography, coupled with flame ionization detector (GCxGC-FID), we have developed a methodology to estimate the total load of HOCs in the lipids of organisms living in a contaminated sediment. Previous research has shown that GCxGC retention times can be used to predict chemical properties such as aqueous solubility and  $K_{ow}$ . With appropriate training set, we have extended this capability to the prediction of the partition constants between the polyethylene sampler and water ( $K_{PEW}$ ) and phospholipids and water ( $K_{PLW}$ ). We then use these partition constants and the relatively constant response factor of the FID, to calculate the corresponding membrane lipid concentration for each compound detected in the GCxGC analysis of a polyethylene sampler equilibrated with the sediment of interest. The total lipid load is then calculated by summing the contributions from individual compounds. We have applied this narcosis calculation to polyethylene sampler extracts from five sites around Boston Harbor. The estimated integral HOCs lipid loads were lower than the proposed narcosis threshold of 0.01g/g of lipid, which was in general agreement with toxicity results from fertilization and embryo development tests. Overall, this analyses of the sediments does not indicate that narcosis toxicities would be expected.

**697 Nutrient Input and Competition of *Phaeocystis* and Diatoms in Massachusetts Bay Spring Bloom** M. Jiang, Univ Of Massachusetts Boston, EOS; D. Borkman, Univ of Rhodes Island; S. Libby, Battelle Memorial Institute; D. Townsend, Univ of Maine; M. Zhou, Univ Of Massachusetts Boston. The phytoplankton community in Massachusetts Bay has displayed dramatic long-term changes and inter-annual variability in the last two decades, with increasing strong *Phaeocystis* (*Phaeocystis pouchetti*) blooms and generally opposite fluctuations in diatom abundances. A new ecosystem model has been developed for Massachusetts Bay to explicitly simulate the *Phaeocystis* dynamics. Model results and an analysis of data collected during the same period suggest that the changes of nutrient conditions play a critical role in controlling the competition between diatoms and *Phaeocystis*. A series of idealized simulations of the model also show long-term trends and inter-annual variability of *Phaeocystis* and diatom biomass that are highly correlated with the observed phytoplankton abundances. These results are further interpreted with the resource competition theory. Using long-term environmental data, we also explore the key drivers for these phytoplankton changes in Massachusetts Bay spring bloom.

**698 The Mechanisms Behind the Decreasing Metal Concentrations in the Surface Sediments of Boston Harbor** L.H. Kalnejais, Univ of New Hampshire, Earth Science Dept; W.R. Martin, Woods Hole Oceanographic Institution, Marine Chemistry and Geochemistry; M.H. Bothner, US Geological Survey, Woods Hole Field Center. The concentrations of silver, lead and copper in the sediments of Boston Harbor have decreased by about



50% between 1978 and 2010. This observation motivated a comprehensive study to understand how geochemical environment, seasonal cycles and sediment resuspension influence the exchange of metals between contaminated sediments, porewater, and overlying bottom water. High-resolution (3 mm) profiles of metals in porewater and sediments were collected at two contrasting sites. The first site, in Boston Harbor, received inputs of contaminants from Boston's sewage outfalls until 2000. The second site, in Massachusetts Bay, is less impacted. At both sites there is a diffusive flux of copper to the water column throughout the year, while silver is released only in winter and lead is never released diffusively. This distinct seasonal pattern is controlled by the competition between processes releasing and removing metals in the shallow porewaters. Metals are released to the porewaters when rapid reactions break down the phases that carry the metals to the sediments and when authigenic sulfides are mixed into the oxic layer and oxidized. On the other hand, dissolved metals are removed from solution by scavenging by iron oxyhydroxides. Deeper in the sediments, dissolved metals are removed by the formation of sulfide minerals. The relative importance of each of these processes differs at each site and between seasons, and determines if there is a diffusive release of metals from the sediments. The release of metals due to sediment resuspension was determined with erosion chamber experiments coupled to hydrodynamic model results. The annual quantity of solid-phase metals resuspended is greater than the river inputs of metals to the Harbor. The dissolved metal release from sediments, due to both sediment resuspension and diffusion is more than 10% of the total (dissolved plus particulate) metal input from rivers. These results indicate that contaminated sediments remain as a continuing and significant source of metals to the water column of the Harbor. We are applying our quantification of metal release mechanisms with data on changing inputs to the Harbor to understand the decreasing metal concentration in the sediments with time. Furthermore, we are continuing to develop models of the long-term behavior of sediment metals and the influence of the sediments on the water quality of Boston Harbor.

**699 Three-dimensional Modeling of Cu and Pb Distributions in Boston Harbor and Massachusetts Bay** L. Li, First Institute of Oceanography, State Oceanic Administration; M. Jiang, Univ of Massachusetts Boston; F. Pala, Battelle; G. Wallace, Univ Of Massachusetts Boston. The transport and distribution of dissolved Cu (Cudiss) and Pb (Pbdiss) in Boston Harbor (BH), Massachusetts Bay (MB) and Cape Cod Bay (CCB) were simulated by integrating trace elements into an existing 3-D hydrodynamic model. Despite cessation of discharge from a major treatment plant in 2000 and concurrent reduction in metal loading, Cudiss and Pbdiss concentrations in the Harbor remained relatively unchanged since the early 1990s. In contrast, the original modeled results in BH only recovered ~ 10% of the observed current standing stock of Cudiss and ~ 20% of Pbdiss in 2002. It is proposed that the previously contaminated sediment of the Harbor may be currently the major source of these metals to the Harbor. Based on the comparison between modeled results and observational data in the Bays, Cudiss can be simulated as a conservatively transported tracer, while a first-order scavenging process needs to be incorporated in the Pb model. Particulate organic carbon (POC) extracted from a water quality model was employed as the sorption and scavenging matrix for particulate Pb. Sensitivity analyses were also conducted to evaluate the importance of each source and sink for Cudiss and Pbdiss in the Harbor and Bays.

**700 Validation and Application of a Chemical Equilibrium Model for the Prediction of Free Copper Ion Activity in Boston Harbor and Massachusetts Bay** F. Pala, Battelle; L. Li, First Institute of Oceanography, State Oceanic Administration; G. Wallace, Univ Of Massachusetts Boston, EOS. The prediction of metal bioavailability in aquatic systems is strictly related with the ability to accurately measure and model free metal ion activity ( $\{Me(n+)\}$ ) and metal speciation in aquatic systems. A Sediment Chemical Equilibrium Model (SCEM) was proposed by Wallace G. to describe the interdependence among ambient  $\{Me(n+)\}$ , surface sediment organic carbon (OCsed), and non-crustal metal sediment ( $Me_{nc}$ ) concentrations in Boston Harbor and Massachusetts and Cape Cod Bays for a variety of metals (Cu, Zn, Pb, Cd, Ag, Hg, Ni). The SCEM interprets the sequestration of metal ions by oxic marine sediment by a surface complexation equilibrium process where sediment OCsed serves as a surrogate for binding ligand concentration, and  $\{Me(n+)\}$ , is linked to OCsed-normalized  $Me_{nc}$  by an apparent adsorption equilibrium constant ( $\beta^*CuSed$ ). The SCEM was validated for the case of Cu by isotherm adsorption titration of slurries of surface (0-1

cm) sediment, collected from various geographic locations and characterized by a range of OCsed and metal concentrations. Interpretation of the isotherm adsorption curves by the SCEM provided an average  $\log \beta^*CuSed$  of  $8.9 \pm 0.2$  (95% CL) and showed no statistical evidence that  $\beta^*CuSed$  changes with sample location or sedimentary organic carbon concentration. The application of the SCEM to Boston Harbor and Massachusetts Bay sediment composition data collected during the past two decades allows for the reconstruction of free copper ion activity, and therefore bioavailability of copper, in the study region.

**701 Analysis of Oral Lead 1, 2, and 3 Pellet Exposure in Northern Bobwhite Quail: A 56 Day Study** R. Gogal, Univ of Georgia, College of Vet Med, Anatomy and Radiology; R. Kerr, J. Holladay, Univ of Georgia, Anatomy and Radiology; L. Tannenbaum, US Army Public Health Command, Environmental Health Risk Assessment Program, US Army Institute of Public Health; B. Meldrum, Virginia Tech, Biomedical Sciences and Pathobiology; S. Williams, Univ of Georgia, Poultry Diagnostics and Research Center; S. Holladay, Univ of Georgia, Anatomy and Radiology. We recently reported in a 28 day study that acute oral lead (Pb) pellet (#9 shot) exposure in Northern Bobwhite quail resulted in significant toxicity in birds ingesting 5 Pb pellets or greater. Birds that received a single lead pellet showed no overt clinical signs of toxicity, but had sustained depressed plasma  $\delta$ -aminolevulinic acid dehydratase (d-ALAD) activity. In the present study, bobwhite quail, fed the same seed-based diet, were orally gavaged once with Pb spent shot (0, 1, 2 and 3 pellets/bird) and evaluated for 56 days for changes in feed consumption, weight gain, peripheral blood Pb levels and physiology, renal, immune and gastrointestinal parameters. Birds in the 3-pellet group showed a significant decrease in weight gain that did not correlate with feed consumption. Mean blood Pb levels increased proportionately to the number of pellets ingested at 7 days post exposure. Similar to the previous study, male blood lead levels were significantly higher compared to the females up to 14 days post exposure. These Pb blood levels declined weekly but remained elevated throughout the study according to baseline data. Similar to the previous study, the  $\delta$ -aminolevulinic acid dehydratase (d-ALAD) activity decreased significantly in all Pb pellet-treated birds, began to recover in the males but remained lower than controls. Blood hematology, immune cytology and the other endpoints were not remarkable. The results of this study support our previous findings with regards to the 1 pellet birds and extend it by showing that birds exposed to 2 and 3 pellets, although becoming transiently moribund, began to show clinical signs of recovery 2 months post exposure.

**702 Arsenic Speciation in Snowshoe Hare (*Lepus americanus*) from Yellowknife, Northwest Territories, Canada** J. Dee, Royal Military College of Canada; I. Koch, K. Reimer, Royal Military. College of Canada, Environmental Sciences Group. Yellowknife, Northwest Territories, is an excellent study site for arsenic speciation in terrestrial mammals because of naturally elevated levels of arsenic in soil as well as local contamination from gold mines in the city. The objective of the present study was to determine arsenic uptake and metabolism in terrestrial animals that are exposed to mostly toxic inorganic arsenic species in their diets. Snowshoe hare (*Lepus americanus*) was targeted because it is a commonly studied ecological receptor, and it is also a potential source of country food to local residents. Hares were collected from contaminated and uncontaminated sites, dissected on site, and their tissues were analysed for arsenic speciation and total arsenic via high-performance liquid chromatography-inductively coupled plasma-mass spectrometry and also using x-ray absorption near edge structure analysis at the Canadian Light Source in Saskatoon, Saskatchewan. Hares collected from contaminated areas had substantially higher arsenic body burdens than hares collected from background areas. Dimethylarsinic acid was the main arsenic species found in tissues from hares collected during spring and early summer periods and arsenite (a toxic form of arsenic) was present in a minor amounts. The formation of DMA in hares is not unexpected and is a metabolite in other mammals, including humans. However we also identified arsenobetaine, a non-toxic arsenic compound with a more complex chemical structure  $[(CH_3)_3As+CH_2COOH]$ , as the major species in one hare (collected in the fall) and as a minor species in two others (one of which was collected in the spring while the other was collected in the fall). Arsenocholine (also non-toxic,  $[(CH_3)_3As+CH_2CH_2OH]$ ) was also identified as a minor constituent in some of the tissues from hares collected during the spring. The arsenobetaine found in the fall-collected hare may have been ingested in mushrooms, which would have been an available food source

during the collection period. Arsenic species in the stomach contents were similar to those in tissues, reinforcing the hypothesis that hares are ingesting the arsenobetaine rather than producing it. Arsenocholine was not identified in the stomach contents but it may be a metabolite of arsenobetaine. Both compounds are excreted readily from mammals in laboratory studies and thus their retention in the hare tissues is unexpected and warrants further research.

#### 703 Direct Uptake and Trophic Transfer of Tungsten into a Gastropod and Plant

**A.J. Kennedy**, US Army Engineer Research and Development Center; J. Lindsay, US Army Engineer Research and Development Center, Environmental Laboratory; A.J. Bednar, US Army Engineer Research and Development Center; D.R. Johnson, US Army Engineer Research and Development Center, Environmental Laboratory; J. Seiter, US Army Corps of Engineers, Research Physical Scientist; R. Boyd, P. Allison, T. Rushing, US Army Engineer Research and Development Center. Tungsten (W) was purported to be a green alternative to lead for munitions, kinetic energy penetrators and canister rounds. However, incidents in Nevada and Massachusetts have led to greater scrutiny on the lack of knowledge-based regulations for W. Previous studies have indicated tungsten in soil bioaccumulates in plant tissue. In the current study, we employed cabbage (*Brassica oleracea*) and a terrestrial snail (*Otala lactea*) to determine the toxicity, bioavailability and food chain mobility of W via direct exposures to contaminated substrate and trophic exposures. Dermal exposure to the more bioavailable tungstate species (WO<sub>4</sub>) indicated more W bioaccumulated in the snail hepatopancreas (152 W/kg) relative to the foot (20 mg W/kg), resulting in a lethal median residue (LR50) of 80 (52 – 123) mg W/kg. A more environmentally realistic exposure to soil spiked with W powder that was aged for four years to allow polymerization (tungstate, polytungstates) was also conducted applying both a concentration gradient of W (range: 0 to >7000 mg/kg) and a single concentration (550 mg W/kg) involving temporal tissue sampling. Results indicated dose-dependent toxicity and bioaccumulation for both cabbage and snails. Kinetic models indicated steady state concentrations of 287 and 34 mg/kg for the cabbage and snail, respectively. Bioaccumulation factors (BAFs) indicated cabbage (BAF = 0.55) bioaccumulated W more efficiently than the snail (BAF = 0.06) through the dermal exposure route. However, snail bioaccumulation efficiency greatly increased (86 mg/kg; BAF = 0.36) when consuming contaminated cabbage. Synchrotron mapping revealed W bioaccumulation in both cabbage veins and leaf tissue and within snail hepatopancreas, mantle and shell nacre. Metal incorporation into the calcium carbonate gastropod shell may advance metals biomonitoring at contaminated sites.

#### 704 Effects of Zinc on Survival and Zinc Uptake in Florida Apple Snails (*Pomacea paludosa*) in an Outdoor Microcosm Study

**T.C. Hoang**, Florida International Univ, Southeast Environmental Research Center/Dept of Environ Study, Loyola Univ Chicago, Center for Urban Environmental Research and Policy; G. Rand, Florida International Univ, Ecotoxicology & Risk Assessment. Zinc (Zn) is an essential element for aquatic organisms. However, depending on water quality, zinc may become toxic at elevated concentrations. Although research has been conducted to characterize Zn uptake and potential transfer through the food chain in aquatic ecosystems, most studies have been conducted with standard test organisms. Florida apple snails (*Pomacea paludosa*) are a key species in the Everglades Ecosystem and the main food source of many higher trophic organisms. To our knowledge, research on Zn uptake in apple snails has not been conducted. Earlier studies in our laboratory concluded that *P. paludosa* accumulated Cu in tissues up to 2800 mg/kg, which may pose a risk to snail predators. The present study characterized Zn uptake in *P. paludosa* in an outdoor microcosm system. In a 100 exposure day study, snails were exposed to five Zn treatment concentrations in 800 L polypropylene tanks that contained 600 L water and 40 L of sediment. Water was spiked with Zn one or two times per week to maintain Zn concentrations. Snails and water samples were collected every two weeks for tissue and water chemistry analyses. Results of this study indicated that Zn affected apple snail survival. After two weeks of exposure, 100% mortality occurred in the highest Zn treatment concentrations (750 µg/L dissolved Zn). The LOEC following 100 days of exposure was 160 µg/L dissolved Zn. Apple snails accumulated high Zn concentrations, especially in the snail viscera. Zn concentrations in the snail foot and viscera increased with exposed time and followed first order kinetics. Zn concentrations in the snail foot and viscera were 858 mg/kg and 23,816 mg/kg dw, respectively. Zn concentrations in the snail shell were less

than 200 mg/kg dw. For snail foot, there was a strong positive correlation for Zn concentrations in water and tissue. However, snail foot tissue Ca and Mg concentrations were negatively correlated with water Zn concentrations. Snail foot tissue Zn concentration was also negatively correlated with snail foot tissue Ca and Mg concentrations. These results indicate that Zn inhibited Ca and Mg uptake in the snail foot tissue. This is in agreement with the literature that Zn inhibits Ca uptake in aquatic organisms. However, inhibition of Zn on Mg uptake has not been demonstrated in the literature. Our earlier study also found that Cu inhibits Mg uptake in apple snails

#### 705 Human Contamination Status and Health Effects of Pb in Lead Battery-Recycle Area, Vietnam

**S.H. Hirata**, Tottori Univ, Dept of Regopnal Environment; K. Komori, F. Horiuchi, Ehime Univ Graduate School for Medicine, Dept of Neuropsychiatry, Neuroscience; T. Noguchi, T. Itai, S. Takahashi, Ehime Univ, Center for Marine Environmental Studies (CMES); P.H. Viet, Hanoi Univ of Science, Vietnam National Univ; L. Khanh, Central Military Hospital 108, Clinical Research Institute 108 for Medical and Pharmaceutical Sciences; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies (CMES). In recent several years, we have monitored Pb contamination in human and environmental samples from a lead battery-recycle area, Dong Mai, Vietnam. In the blood samples of a study in 2007, mean Pb level in Dong Mai people was found to be 31.1 µg dL<sup>-1</sup> (normal range: < 10 µg dL<sup>-1</sup>). Blood Pb levels in more than 95% of the donors exceeded 10 µg dL<sup>-1</sup>, which can cause mild toxic symptoms such as decline of heme-synthase activity. In addition, Pb levels in about 50% of the donors exceeded 40 µg dL<sup>-1</sup>, which can manifest neurobehavioral disorders. These results suggest that serious Pb pollution exists in Dong Mai. In 2010 sampling, to evaluate health effects on adults in Dong Mai, we executed Mini-Mental State Examination (MMSE), a neurobehavioral test, and found that orientation, long-term memory, attention and concentration in Dong Mai people were defective than in other areas. Moreover, we took MRI images of two adult men whose blood Pb levels were 107 µg dL<sup>-1</sup> and 75 µg dL<sup>-1</sup>. In these MRI findings, encephalopathy due to Pb poisoning was noticed in the donor with 107 µg dL<sup>-1</sup> level. In order to assess health effects on children in Dong Mai, we took hair, blood and urine samples from them, and executed psychopathological test (Strength and Difficulties Questionnaire: SDQ) in the survey conducted in 2011. Total SDQ score in Dong Mai children was higher than in the general trend in Vietnam and other countries. This result indicates that disorders in behavior are more serious in Dong Mai children than in others. The present study clearly revealed that pollution related diseases by Pb do exist in Dong Mai, and health effects by Pb poisoning on people, particularly children are apprehensive. Continuous monitoring and risk assessment of the people, epidemiological research, and measures to improve the labor environment for the workers and people are necessary in Dong Mai.

#### 706 Including Metal Speciation in LCA: Choosing the Appropriate Regionalization Scale

**G. Plouffe**, Ecole Polytechnique de Montreal, CIRAIG; C. Bulle, CIRAIG, Ecole Polytechnique de Montreal, Chemical engineering Dept; L. Deschenes, CIRAIG, Chemical Engineering. Ecotoxicological impacts are sometimes difficult to consider in life cycle assessment (LCA) because they affect local environments surrounding emission sources and thus require data not always available. This is particularly true for metals, because their toxicity depends largely on speciation, which in turn, is closely related to physico-chemical properties of the environment. Recently, the use of freshwater archetypes has been suggested to account for the influence of freshwater physico-chemical properties on metal speciation in LCA. Although really interesting, this approach is not necessarily applicable to soils because soils heterogeneity is much greater than for freshwater environments. The most influent soil physico-chemical characteristics often covary and are not easily definable. The main goal of this project is thus to determine a regionalization scale for soils that encompass the local parameters influencing metal speciation and large enough to be applicable to a global method like LCA. The Harmonized World Soil Database version 1.1 (Nachtergaele et al., 2009) was used to define soil archetypes according to five influent soil physico-chemical parameters on metal speciation, chosen on the basis of a literature review. The selected parameters are: soil pH, soil texture (clay and sand content), organic and inorganic carbon contents. Intervals for each soil parameter were created and each archetype was attributed a combination of these intervals. For example, archetype 1 includes all soils with low pH, low clay, low sand, low inorganic and low organic carbon contents. The definition of soil archetypes is validated

using at first commercially available speciation models (WHAM 6.0 and MINEQL+) and will be confirmed with a new speciation model developed specifically for soils in this project. Using the new regionalization scale here defined, new characterization factors with quantified spatial variability will be obtained for Zn, Ni and Cu for terrestrial ecotoxicity. Preliminary results show that it is possible to group soils of the world in forty-two archetypes and account for five soil physico-chemical parameters (pH, clay and sand content, organic and inorganic carbon), a scale that could be adequate for its implementation in LCA.

**707 Metallomics of Wildlife: An Approach to Understand the Mechanism of Mercury Detoxification** T. Agusa, Dept of Legal Medicine, Shimane Univ Faculty of Medicine, Shimane Univ, Center for Marine Environmental Studies (CMES), Ehime Univ, Ehime Univ; T. Ikemoto, Center for Marine Environmental Studies (CMES), Ehime Univ; T. Kunito, Shinshu Univ; S. Yasugi, S. Tanabe, H. Iwata, Center for Marine Environmental Studies (CMES), Ehime Univ. Metallomics is developing as an interdisciplinary science to understand the roles and functions of biomolecules that bind to trace elements in biological systems. This field covers chemical speciation, dynamics, and kinetics of trace elements, and is expected to unveil the physiological and pathological mechanisms related to trace elements in animal health and disease. We have investigated Hg accumulation and its detoxification mechanism in marine mammals and seabirds by the approach of metallomics. A significant positive correlation between Hg and Se was observed in the liver of these animals. Concentrations of Hg and Se in the hepatic nuclear/lysosomal/mitochondrial fraction were higher than those in the microsomal and cytosol fractions. With an increase in the concentration of Hg in the nuclear/lysosomal/mitochondrial fraction, extraction efficiencies of both elements by protein extraction agents were decreased and the molar ratio of Hg/Se was close to 1.0, suggesting the formation of Hg-Se complex in a Hg concentration-dependent manner. In the cytosol, a large portion of Hg and Se was detected in the high molecular weight fraction. The equimolar of Hg and Se was observed in the high molecular weight fraction of the liver cytosol that showed high Hg levels, implying that the Hg-Se complex may bind to high molecular weight substances. Further metallomics approach may provide an insight into the mechanism of Hg detoxification.

**708 Predicting Exposure of Bat to Soil-associated Heavy Metals** B. Hernout, Univ of York, Environment; K. Somerwill, FERA; K. Arnold, C. McClean, Univ of York; V. Grimm, Helmholtz Center for Environmental Research; A. Boxall, Univ of York. Wildlife may be exposed to heavy metals in soils via uptake through the food chain. The accumulated metals may then affect the health of the wildlife species. In this study, we developed a modeling framework to predict the exposure of 14 bat species in the UK to soil-associated heavy metals (Pb, Cu, Zn, Cd) and to determine the potential risks of exposures to bat health. The modeling framework considered exposure to single metals as well as metal mixtures. Our results show that highest exposure occurs around industrial areas, where predicted exposures for several bat species were at a level at which toxicological effects might be expected. Lead was found to be the biggest contributor to risk. A sensitivity analysis of the modeling framework is currently underway and the exposure component of the modeling framework is currently being evaluated using analysis of bat carcasses obtained from a large number of sites distributed across England and Wales. The presentation will explain the model, the results of simulations to date and the findings of the sensitivity analysis. Initial validation data will also be presented.

**709 Study of Biodegradable Solvents for the Biorefinery** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences. A wide range of approaches have been investigated for manipulation and conversion of biomass. Of paramount importance in all these methods is the choice of a suitable solvent, (including scCO<sub>2</sub>, water, or ionic liquids). Due to the large volumes of solvent required in a commercially viable biorefinery, release into the environment is inevitable, whether from vented gasses, water waste, solvent leaching or accidental spills. Our research investigates the development of biodegradable ionic liquids for the biorefinery. Results towards the synthesis of low antimicrobial toxicity and ecotoxicity ionic liquids is presented. While all efforts to recycle solvents are made within the biorefinery, a 'fail-safe' where the ionic liquid on release biodegrades is a worthwhile goal.

**710 The Effect of Different Lignocellulosic Pretreatment Methods on Diverse Microbial Consortia in a Bioprocess Step to Generate Fuel Precursors** L.T. Angenent, M.T. Agler, J.J. Werner, Cornell Univ, Biological and Environmental Engineering; S. Heger, Institute for Environmental Research, RWTH Aachen Univ, Institute for Environmental Research, RWTH Aachen Univ, Dept of Ecosystem Analysis; H. Hollert, RWTH Aachen Univ, Institute for Environmental Research, Dept of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen Univ, Department of Ecosystem Analysis, RWTH Aachen Univ, Inst. for Environmental Research; B.S. Dien, L.B. Iten, M.A. Cotta, NCAUR-ARS-USDA, Bioenergy Research Unit. We used diverse microbial consortia to convert lignocellulosic biomass into short-chain or medium-chain carboxylates, which are important future platform chemicals for bioenergy production. We derived our consortia from natural sources, and then evolved them via applied environmental conditions to either produce C4 (n-butyrate), C6 (n-caproate), and C8 (n-caprylate) carboxylates. The lignocellulosic feedstocks that are used as substrates must be pretreated to destroy the lignocellulosic matrix with the goal to speed up hydrolysis by the microbes. Within an experimental design that entailed four 5-L bioreactors we tested the effect of three different chemical/physical pretreatment methods (and no pretreatment as the control) on the biological conversion of corn fiber (i.e., a waste product from the corn kernel-to-ethanol industry) into n-butyrate. The three pretreatments were performed in fluidized sand bed reactors at 36% corn fiber w/v in water at 160°C for 20 min with the variations: 1. 0.5 % w/v H<sub>2</sub>SO<sub>4</sub> (acid); 2. 1:10 CaO to biomass ratio (base); or 3. hot water only. We performed massively parallel pyrosequencing to examine microbial community dynamics and to link environmental conditions with community changes over the entire 450-day operating period in intervals of < 3 weeks. In addition, we performed an ecotoxicological evaluation by different acute and mechanism specific in vitro biotests. We found differences between the pretreatment methods in regards to their ecotoxicity levels for the resulting biomass solution, which is the substrate for our bioreactors. The pretreatment methods also had a profound effect on the community composition of the microbial consortia in the bioreactors.

**711 What's Up Inside the Reactor – Biotests for Risk Assessment of Biofuel Fermentation** S. Heger, Institute for Environmental Research, RWTH Aachen Univ, Institute for Environmental Research, RWTH Aachen Univ, Dept of Ecosystem Analysis; K. Bluhm, M. Brinkmann, K. Winkens, A. Schneider, M. Wollenweber, S. Maletz, Institute for Environmental Research, RWTH Aachen Univ; M.T. Agler, Dept of Biological and Environmental Engineering, Cornell Univ; T. Seiler, Institute for Environmental Research, RWTH Aachen Univ; L.T. Angenent, Dept of Biological and Environmental Engineering, Cornell Univ; H. Hollert, Institute for Environmental Research, RWTH Aachen Univ. As a consequence of the increasing use of biofuels and bioenergy as alternative energy sources to replace fossil fuels, there is an urgent need for ecotoxicological evaluation, in particular within processes that are used to gain these alternative energies. Although research in the field of energy sciences was steadily growing during the last years, ecotoxicological data on the intermediates in the fermentation processes have not yet been sufficiently investigated. Thus, a project was initiated to assess the pollutant development by microbial conversion from substrate to end product in cooperation between Cornell Univ and RWTH Aachen Univ. Cornell Univ works on the conversion of lignocellulosic wastes into n-butyrate, which can be further converted to the liquid biofuel n-butanol in a subsequent fermentation step. To ensure sufficient biological breakdown, lignocellulosic materials are pretreated with several different techniques, such as hot water acid and hot water base treatment. This process, however, has a known disadvantage of generating toxic compounds, which can inhibit microbial cultures that are grown in down-stream bioprocesses. Undefined mixed cultures may destroy the toxic compounds, which would prevent inhibition of these microbial cultures. However, if intermediates are not degraded they are assumed to be of ecotoxicological relevance due to possible release into the environment. In this approach, we want to meet this hypothesis applying a battery of acute and mechanism specific in vitro biotests. Biological analysis will focus on different pretreated and untreated substrate samples, as well as on complementary effluent samples. In detail, cytotoxicity will be assessed using the neutral red retention assay, and Ah receptor agonist activity will be detected with the EROD assay, both using RTL-W1 cells. In addition endocrine activity will be determined in the yeast endocrine screen (YES) assay and embryotoxicity by means of the fish embryotoxicity test (FET). Further biotests are considered.



Finally, toxic impacts of effluents resulting from the production process will be assessed.

**712 Chemical and Ecotoxicological Analyses of the Water-soluble Fractions of Neat Biodiesel, Diesel and Biodiesel/Diesel Blends** S.A. Pereira, Federal Univ of Bahia, Dept of Biology; V.Q. Araujo, Federal Univ of Bahia; M. Reboucas, F. Vieira, M. Almeida, Braskem; F. Chinalia, Cranfield Univ; I.A. Nascimento, Federal Univ of Bahia, Dept of Biology. Biodiesel is considered less toxic than diesel, but the comparison is mostly based on gaseous emissions. Little information is available regarding the potential toxic effects of different biodiesel/diesel blends when reach water bodies and/or groundwater. The aim of this study is to evaluate and compare the toxic properties of the water-soluble-fraction (WSF) obtained from diesel, biodiesel and biodiesel/diesel blends. The hypothesis is that biodiesel is less toxic than diesel, imposing, therefore, less impact in the case of environmental contamination after leaks and/or spills. Aromatics are present in the diesel-WSF. This study showed that their concentration correlated positively with increasing ratios of diesel. On the other hand, biodiesel can interact with any aqueous matrix and generate methanol, which is toxic. Highest methanol concentration was observed in biodiesel-WSF, but little concentrations were also found in blends. WSF toxicity tests were carried out using marine and freshwater microalgae. Diesel alone showed to be more toxic than all the tested blends and biodiesel. Depending on the tested blends and microalgae species, the WSF concentrations inhibiting culture growth in 50% (IC50), varied from 6.6 to 81.4%. Methanol showed to be toxic, but seemed to not synergistically increase the toxic effect of aromatics in the WSF. The results support the hypothesis that blending biodiesel with diesel is an effective strategy for reducing environmental impact caused by accidental spills or leakages.

**713 Two Potential Biofuels in an Ecotoxicological Investigation** K. Bluhm, Institute for Environmental Research, RWTH Aachen Univ, Department of Ecosystem Analysis, Instit.for Environm.Research, RWTH Aachen Univ., Dept of Ecosystem Analysis; S. Heger, Institute for Environmental Research, RWTH Aachen Univ; J. Loerks, T. Seiler, Institute for Environmental Research, RWTH Aachen Univ, Dept of Ecosystem Analysis; A. Schaeffer, Institute for Environmental Research, RWTH Aachen Univ, Chair of Environmental Biology and Chemodynamics; H. Hollert, Institute for Environmental Research, RWTH Aachen Univ, Department of Ecosystem Analysis. The interest in biofuels for the transport sector increased within the last years. Biofuels are considered renewable alternatives with the benefits of reduced dependence on fossil fuels and a potential to slow down the effect of global climate change due to decreased greenhouse gas (GHG) emissions. Consequently, biofuel production increased dramatically in recent years. Growing production capacities and the associated rise in biofuel consumption also increased the risk of a release into the environment. However, comparatively little knowledge on (eco)toxicological effects of biofuels are available. In this study we investigated the potential risks of selected substances derived from biomass and with promising properties for the use in combustion engines (ethyl levulinate and 2-MTHF). The substances were compared to water accommodated fractions of a fossil diesel fuel using assays on the acute aquatic toxicity and biotests to reveal mechanism-specific effects. First results revealed practical problems with the investigation of these materials (e.g., due to their low pH-value and rapid corrosion of pipette tips and microplates) as well as indications for adverse impacts on the organisms or cell cultures tested. 2-MTHF revealed a lower toxic potential in the fish embryo toxicity (FET) test with zebrafish and the cytotoxicity test using RTL-W1 cells than ethyl levulinate. In addition, a statistically significant effect on the development of the head length in zebrafish embryos was observed for ethyl levulinate even at 40 mg/L, the lowest test concentration. The Ames fluctuation assay gave no mutagenic potential for both 2-MTHF and ethyl levulinate. In conclusion, the results reveal a potential hazard of these substances and further investigations of potential biofuels or biofuel components are essential to provide a basis for identifying alternative fuels with low environmental impact. Acknowledgement: This work was performed as part of the Cluster of Excellence "Tailor-Made Fuels from Biomass", which is funded by the Excellence Initiative by the German federal and state governments to promote science and research at German universities.

**714 Understanding Resilience in Coupled Industrial-Ecological Systems** T. Seager, Arizona State Univ, School of Sustainable Engineering & Built Environm; J. Park, P.C. Rao, Purdue Univ; D. Mu, U Minnesota,

Twin Cities. The recent investment boom and collapse of the corn ethanol industry calls into question the long-term sustainability of traditional approaches to biofuel technologies. Compared with petroleum based transportation fuels, biofuels production systems are more closely connected to complex and variable natural systems. Especially as biofeedstock production itself becomes more independent of fossil fuel-based supports, stochasticity will become an increasingly important inherent feature of biorefinery systems. Accordingly, a fundamental change in design philosophy is necessary to ensure the long-term viability of the biorefinery industry. To respond effectively to unexpected disruptions, the new approach will require systems to be designed for resilience, as well as for risk. However, quantitative tools for understanding the resilience of alternative fuel technologies are poorly developed, if available at all. This presentation describes the distinction between risk management and resilience approaches, especially as applied in the context of the biorefinery supply chain, and proposes a novel approach to assessing the resilience of biorefinery systems in terms of their stability to exogenous shocks.

**715 Fitness for Purpose in Dioxin Testing** Y. Tondeur, B. Vining, J. Hart, Analytical Perspectives. Achieving a reasonable balance between the cost of making incorrect decisions and testing is an outgrowth of the performance-based measurement system (PBMS) introduced by EPA to provide more flexibility, and to facilitate the incorporation of new technologies, as well as the latest knowledge and improvements for analytical protocols. The latter were promulgated decades ago under a command and control environment. Over time, it became evident that changes were necessary to dislodge the performance status quo. An analytical result is deemed fit for purpose when its uncertainty maximizes its expected utility. In essence, achieving a smaller uncertainty in a measurement lowers the chance of making an incorrect decision or incorrectly assessing compliance. Existing analytical protocols, as well as the current ways of doing business, are not serving the end user well – because fitness for purpose is not central and none seem to be aware that the data quality objectives associated with analytical measurements are constantly in flux. Our solution to EPA's direction was to develop a novel protocol which, when used in conjunction with existing methods, transforms the infrastructures of current methodologies by aligning them with PBMS. The innovative approach ensures quality improvements, the generation of effective data, and the removal of delays associated with the promulgation of revised methods. The new protocol is the product of 10 years of work, and is supported by extensive validation. The approach does the following: 1) fundamentally renders PBMS tangible, 2) allows a comprehensive and accurate assessment of performance, 3) acts as a gate keeper for performance and data quality before the data reaches the end user, 4) offers uncertainty measurements for each toxic target analyte and for the TEQ on a per sample basis (i.e., when & where needed!), 5) provides a context for assessing data quality and for making intelligent and rational decisions, 6) captures information for traditional lab data flags by quantifying their impact on data reliability, and 7) is a tool for planning future testing events such that the data generated are fit for purpose (i.e., meet the end user's data quality objectives and are found to lie within the tolerable error range necessary for decision making). Such an approach is particularly important at this point in time since action limits have been set low and treacherously close to actual detection limits.

**716 Analysis of Dioxins, Furans, and Polychlorobiphenyls in Fish and Clams for the Houston Shipping Channel, TX** B. Subedi, Baylor Univ, Dept of Chemistry and Biochemistry, Baylor Univ, Dept of Biology, Baylor Univ, Dept of C; L. Aguilar Lazaro, Baylor Univ, Environmental Science; S. Usenko, Baylor Univ, Dept of Environmental Science, Baylor Univ, Assistant Professor. Fish and clams were collected from the San Jacinto River waste pits, a superfund site in Houston, TX and analyzed for polychlorodibenzo-*p*-dioxins, polychlorodibenzofurans (PCDD/Fs) and dioxin like polychlorobiphenyls (dl-PCBs). Sample preparations comprised of tissue homogenization, simultaneous pressurized liquid extraction and cleanup, and concentration. Isotopically labeled surrogates were spiked prior to extraction and were used to correct for target analytes loss during sample preparation. Samples were analyzed employing gas chromatography negative chemical ionization mass spectrometry. The method detection limits ranged from 2.0 to 10 pg/g ww in fish tissues. Ten out of twelve high priority dl-PCBs (Identified by World Health Organization, 2005) were measured with concentrations ranged from 2.0 to 948 pg/g ww. Concentrations of three most abundant dl-PCBs (PCB 105, 118, and 123) were an order of

magnitude higher in fish than in clam tissue. The toxicity equivalent calculated using toxicity equivalency factor ranged from 0.46 to 0.80. PCDD/Fs were not detected in fish tissues; however, 2,3,7,8-TCDF was measured up to 30 pg/g ww in clam tissues.

**717 Semi-continuous Determination of PCDDs/PCDFs and dl-PCBs in Stack Emissions: Policy Development and Laboratory Strategies** L. Rivera-Austrui, CSIC, Dioxin Laboratory – Environmental Chemistry Dept; K. Martinez, CSIC; E. Abad, M. Abalos, CSIC, Dioxin Laboratory – Environmental Chemistry Dept; M. Adrados, J. Rivera, J. Saulo, CSIC. At present, dioxin stack emission determinations in Europe are set according to the European Standard EN1948:1(2007) based on manual short-time samplings of 6 hours. European Directive 2000/76/CE on waste incineration sets the dioxin emission limit for stacks (0,1 ng I-TEQ/Nm<sup>3</sup>) and refers to this standard to verify the conformity of plant emissions twice a year. A part from manual method spot measurements, automatic sampling systems, which have been used since several years, are capable of permanently sampling the stack and allow semi-continuous determination (four weeks sampling and analytical determination in the laboratory). Continuous sampling systems allow the measurement of total plant emissions for longer periods, providing reliable data that is a useful tool for the improvement of plant operation protocols and to obtain accurate results that can considerably improve the current emission inventories. This has led to their generalized use due to the interest of plant administrators, or because of local or national legal requirements (e.g., Belgium, France, Italy). At the same time, tests have been conducted to certify sampling systems (EPA, TUV, MCert) or in some cases, to validate these methods by intercomparison measurements with manual sampling methods. However, little information has been published on the analytical treatment in the laboratory of these samples which are mainly characterized by a far higher amount of sampled volume resulting in higher amounts of analytes of interest but also interfering compounds. In this study are presented the results of several tests developed in order to extend the scope of validation of the methodology for the analysis of samples provided by continuous sampling systems. These tests were carried out with samples collected with automatic isokinetic sampling system in a monthly basis from 4 different industrial processes (cement plant with and without waste combustion, municipal and hazardous waste combustor). Special attention is paid to parameters that differ from the well-established methodology described in EN1948 or EPA23, such as levels of native and labelled analytes when using an aliquot, sampling recoveries related to longer periods, etc. From these experiences, a scheme of aliquot and standard addition protocol is suggested. The presented experiences and results can help to set the basis for analysis methodology to include in future standards concerning long-term sampling determinations.

**718 Are Dioxins Dechlorinated in Sewers?** L. Rodenburg, Rutgers, the State Univ of New Jersey, Dept of Environmental Sciences; J. Guo, Rutgers, the State Univ of New Jersey, Dept of Environmental Science; S. Du, N.U. Oseagulu, RUTGERS Univ; D. Fennell, RUTGERS Univ, Dept of Environmental Sciences. Recently we published evidence that polychlorinated biphenyls (PCBs) undergo extensive microbial dechlorination in sewers, especially combined sewers. Since PCBs and polychlorinated dibenzo-*p*-dioxins and -furans (PCDD/Fs) are structurally similar, we hypothesized that PCDD/Fs might also be dechlorinated in sewers. To test this hypothesis, we used Positive Matrix Factorization to analyze several databases on concentrations of PCBs and PCDD/Fs in the influents and effluents of more than 70 wastewater treatment plants in New Jersey, New York, Pennsylvania, and Delaware. Most of the samples of both influents and effluents contain evidence of dechlorination of PCBs via either a partial dechlorination regime that produces primarily PCBs 47 and 51, or an advanced dechlorination regime that produces primarily PCB 4. Furthermore, analysis of a data set containing information on both PCBs and the 17 2,3,7,8-substituted PCDD/Fs indicates that the advanced dechlorination signal that is dominated by PCB 4 is also characterized by high proportions of 1,2,3,4,6,7,8- heptachlorodibenzo-*p*-dioxin (HpCDD), a documented dechlorination product of octachlorodibenzo-*p*-dioxin (OCDD). OCDD was absent in this advanced dechlorination factor, suggesting that OCDD is dechlorinated to 1,2,3,4,6,7,8-HpCDD in sewers, raising the possibility that PCDD/Fs may undergo dechlorination via a number of pathways in sewers.

**719 Sequence and In Vitro Function of Chicken, Ring-necked Pheasant and Japanese Quail AHR1 Predict In Vivo Sensitivity to Dioxins** R. Farmahin, Univ of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre; D. Wu, D. Crump, Environment Canada, National Wildlife Research Centre; J.C. Herve, Univ of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre; S.P. Jones, Environment Canada, National Wildlife Research Centre; M.E. Hahn, Woods Hole Oceanographic Institution; S.I. Karchner, Woods Hole Oceanographic Institution, Biology; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; S.J. Bursian, M.J. Zwiernik, Michigan State Univ, Dept of Animal Science; S.W. Kennedy, Univ of Ottawa, Dept of Biology, Environment Canada, National Wildlife Research Centre. There are large differences in sensitivity to the toxic and biochemical effects of dioxins and dioxin-like compounds (DLCs) among vertebrates. Some of the largest differences in sensitivity to aryl hydrocarbon receptor-mediated effects of DLCs occur among species of birds. Avian toxic equivalency factors (TEFs) have been established by the World Health Organization and are applied in the risk assessment of mixtures of DLCs including polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and some polychlorinated biphenyls (PCBs). However, TEFs do not account for differences in species sensitivity to DLCs, and may not accurately predict DLC toxicity in all avian species. Previously, we demonstrated that differences in sensitivity between domestic chicken (*Gallus gallus domesticus*) and common tern (*Sterna hirundo*) to aryl hydrocarbon receptor 1 (AHR1)-dependent changes in gene expression following 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure is based upon the identities of the amino acids at two sites within the ligand binding domain of AHR1 (chicken: Ile324\_Ser380, highly sensitive vs. common tern: Val325\_Ala381, less sensitive). Here, we tested the hypotheses that (a) the sensitivity of other avian species to TCDD, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and tetrachlorodibenzofuran (TCDF) is determined by the amino acids at sites corresponding to 324 and 380 and (b) Ile324\_Ala380 and Val324\_Ser380 genotypes confer intermediate sensitivity to DLCs in birds. We compared ligand-induced transactivation function of full-length AHR1s from chicken, common tern, ring-necked pheasant (*Phasianus colchicus*; Ile324\_Ala380) and Japanese quail (*Coturnix japonica*; Val324\_Ala380) and three Japanese quail AHR1 mutants. The results suggest that avian species can be grouped into three general classes of sensitivity to DLCs. Both AHR1 genotype and in vitro transactivation assays predict in vivo sensitivity. Contrary to the assumption that TCDD is the most potent DLC, PeCDF was more potent than TCDD at activating ring-necked pheasant (5-fold), Japanese quail (23-fold) and common tern (31-fold) AHR1. Our results are consistent with previous in vitro and in vivo work that demonstrated ligand-dependent species differences in AHR affinity. The findings and methods will be of use for dioxin and DLC risk assessments.

**720 Unintentionally Produced Persistent Organic Pollutants (POPs) from Solvent Production** R. Weber, POPs Environmental Consulting; F. Oliaci, Cambridge Environmental, LLC. The Stockholm Convention currently list five Persistent Organic Pollutants (POPs) as unintentionally produced POPs (UPOPs): polychlorinated dibenzo-*p*-dioxins (PCDD) polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCB), hexachlorobenzene (HCB) and pentachlorobenzene (PeCB). These UPOPs are commonly formed as by-products during the production of chlorinated organics; in processes where elemental chlorine is present; and in thermal processes in the presence of all forms of chlorine. For certain solvent productions (e.g., tetrachloroethene, trichloroethene, tetrachloromethane, dichloroethene) it is known that large amounts of hexachlorobenzene (HCB) waste was and is produced. In some cases these wastes have been deposited and have generated POPs contaminated sites. Recently it has been discovered that some of these wastes also contain relevant levels of POPs PeCB. Furthermore also hexachlorobutadiene (HCBD) – nominated in May 2011 by the EU for assessment as POPs under the Stockholm Convention – is included in such wastes. This contribution gives an insight in some contemporary case studies and related contaminated sites or stockpile management challenges. Such historic deposits are probably now the major stocks of HCB, PeCB and HCBD. The likely contamination of the wastes with other UPOPs is also discussed. As a consequence of the formation chemistry it is likely that chlorinated styrene (octachlorostyrene), chlorinated naphthalene and chlorinated biphenyls and depending on the oxygen and water content possibly also PCDF/PCDD can be expected in some or

all such deposits and contaminated sites. The case studies demonstrate that production of key chlorinated solvents in factories without adequate waste destruction capacity generated thousands of tonnes of hazardous waste containing high levels of the UPOPs HCB and PeCB. The study highlights the importance of a more detailed assessment of these deposited wastes from chlorinated solvents productions and the need of developing inventories of current productions and existing stockpiles and thus support future assessment and clean-up of these sites.

**721 Policy Implications from Observations of PCBs and PCDD/Fs Fate, Transport, and Inter-media Distributions** N.L. Howell, H.S. Rifai, Univ of Houston, Civil & Environmental Engineering. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are highly chlorinated, lipophilic, persistent pollutants composed of hundreds of chemical variants. Only 29 are considered to be toxicologically relevant as they relate to what is often touted as "the most toxic man-made chemical" (2,3,7,8-TCDD). In this research, a comparative analysis was undertaken of geographic distributions, observed bioaccumulative ability, medium-to-medium transfer, and potential sources of PCDD/Fs and PCBs for 1041 samples of bed sediment, suspended sediment, water, catfish, and blue crab in the Houston Ship Channel (HSC) in Texas over a three-year period. Important findings include that current and historical sources in the HSC have created different trends in the contaminants that seem to correlate to known Superfund site locations, that water-phase partitioning between the contaminants are different (not always found primarily on suspended sediments), and that bioaccumulation was more effective in PCBs over PCDD/Fs making PCBs more toxicologically relevant than what toxic potency alone would suggest. A characterization of bioaccumulation factors (BAFs) using various water column contaminant partition models and estimates of the highly hydrophobic sorbent, black carbon, was undertaken. The observed variation in BAFs and the partial explanation of that variation using abiotic contaminant concentration and ancillary measurements (e.g., mass suspended solids, particulate organic carbon, etc.) reduced the total observed variation in biotic concentrations and allowed better prediction of what concentrations will be. A key finding is that more of the congeners than the 29 "TCDD-like" are useful for understanding the source and fate-and-transport issues related to actualized toxicity. For example, the use of chemical fingerprinting may aid in locating sources and tracking their effects. Safe concentrations in fish and crab are the goals for the eventual lifting of consumption advisories, but the biota may not be an adequate target for the prediction of future human health risk. Complex geographic distributions and nuanced fractionation amongst abiotic phases in the water help determine the biotic endpoint and thus must be used to explain known aquatic biota contamination and predict what will occur if a water body is left on its own or if remediation is attempted.

**722 Soil Cleanup Levels for Dioxins: Contrasting a Site-specific Analysis with the Draft EPA PRG** L. Yost, Exponent; C. Kirman, Summit Toxicology; R. Budinsky, J. Zabik, The Dow Chemical Company. The 2010 draft EPA preliminary remediation goal (PRG) for chlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs as toxicity equivalence TEQD/F) in residential soil is 13-fold lower than the prior value of 1 ppb used nationwide. PRGs, while not cleanup standards, are often applied as such or require substantial resources to characterize potential exposure and risk in efforts to establish alternative values. This presentation will provide an overview of the use of problem formulation as described in the National Academy of Sciences (NAS) report titled *Science and Decisions: Advancing Risk Assessment*, (i.e., the Silver Book) as a critical first step to derive a range of site-specific soil cleanup levels for PCDD/Fs. Estimates were derived through deterministic and probabilistic methods and integrated considerable site-specific data on influential exposure terms (residence duration, bioavailability, and exposure frequency), and applied soil ingestion rate data for children and adults from recent studies. The problem formulation approach then allows for incorporation of site-specific biomonitoring data to further refine risk estimates and to improve risk management. Derivation of toxicity values for TEQD/F representative of human health risk is still underway with EPA responding to extensive comments from the EPA Science Advisory Boards and the NAS on the proposed EPA Draft Dioxin Risk Assessment. Analyses here applied toxicity values derived from NTP (2006) assay with the intent to address NAS recommendations regarding cancer risk assessment. Deterministic soil cleanup level estimates ranged from 19 to 250 ppb through application of linear and nonlinear cancer toxicity values, and a cleanup level of

5.3 ppb was estimated based on the World Health Organization toxicity value for noncancer and cancer endpoints. A wide range of cleanup level estimates were calculated using probabilistic methods, with the prior EPA 1 ppb clean-up value falling below the first percentile of estimates, suggesting that the 1 ppb value is health protective. Supporting these estimates are the extensive biomonitoring studies that show little relationship between human contact with dioxin in soil and dioxin in human serum (i.e., the Univ of Michigan Dioxin Exposure Study, and studies in Japan, and West Virginia). The problem formulation approach provided a multiple-lines-of-evidence basis supporting informed risk management decisions that are health protective and practical.

**723 Chemistry and Toxicity of Urban Stormwater: Overview** G.S. Toor, Univ of Florida, Gulf Coast Research & Education Center, Gulf Coast Research & Education Center, Univ of Florida, Soil & Water Science Dept; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. Urbanization of our planet has been an ongoing phenomenon and a necessary evil to accommodate increasing populations. Urban growth alters the natural processes and pathways of water and pollutant transport in human-dominated watersheds. The foremost impact of urbanization is increase in impervious surfaces (building rooftops, parking lots, paved streets) that reduce water percolation in soil and increase the amount and intensity of stormwater runoff. As stormwater flows over the impervious surfaces, it carries pollutants such as sediment, pesticides, hydrocarbons, and trace metals from land and rapidly transport them to nearby water bodies. The goal of this presentation is to provide (1) an overview of common sources of pollutants found in urban stormwater runoff and their fate and transport to water bodies and (2) discuss the toxicity, bioaccumulation, and trophic transfer potential of stormwater pollutants to aquatic organisms.

**724 Recent and Ongoing Developments in the Transport, Occurrence, Fate and Ecotoxicology of Pyrethroids in Urban Settings** A.C. Barefoot, E.I. Du Pont De Nemours And Co., Inc., Crop Protection Products; P. Hendley, Syngenta Crop Protection, LLC., Senior Syngenta Fellow; M. Dobbs, Bayer Crop Science; K. Henry, Syngenta Crop Protection, LLC, Ecological Sciences; R. Jones, Bayer Crop Science; G. Hancock, Waterborne Environmental, Inc., Ecotoxicology. Over the last few years, considerable interest has developed in the occurrence and potential impacts of pyrethroid insecticide residues found in sediment and water samples from urban streams. A great deal of related monitoring has taken place in California where the Dept of Pesticide Regulations instituted a re-evaluation program to ensure development of data on pyrethroids to help refine related risk assessments and identify the most likely residue sources and, where necessary, potential mitigation alternatives. The Pyrethroid Working Group (PWG) has been developing data to address the re-evaluation requests in addition to conducting a wide range of related studies to better understand the science underlying the transport, occurrence and impact of the pyrethroids arising from residential uses. This presentation will use the conceptual model that has been developed for pyrethroid occurrence and transport data from a CA-wide use survey to provide a context to help explain how the studies already completed or underway are refining our knowledge. Some of the studies are "real world" monitoring programs in several stream systems matched with bioassessment studies and sediment deposition area mapping in the same streams. Laboratory-based studies include sediment fate studies, acute ecotoxicology studies on *Hyaella azteca* and *Chironomus tentans*, as well as ongoing improvements in analytical methods. In addition, indoor studies have been conducted to examine the washoff potential of commercial pyrethroid products from various building surfaces. A field site has also been established for conducting replicated studies to identify the transport pathways which make the most significant contributions to residential pyrethroid transport (e.g., driveways vs. vertical walls vs. turf). Preliminary results and future plans for these studies will be discussed.

**725 Pyrethroid Product Washoff from Driveway Concrete Under Simulated Rainfall Conditions** P. Hendley, Syngenta Crop Protection, LLC., Senior Syngenta Fellow; R. Jones, Bayer Crop Science; G. Hancock, Waterborne Environmental, Inc., Ecotoxicology; C. Harbourt, Waterborne Environmental Inc. A study has been conducted to determine the potential for washoff of a series of commercial pyrethroid insecticides following application to residential concrete surfaces such as driveways. Small concrete slabs (24 inch by 9 inch) were poured and brushed to simulate a driveway



concrete surface. After the slabs had been aged for several weeks, individual slabs were each treated with one of 17 different commercial pyrethroid insecticides. The products were applied using a track sprayer at use rates and spray volumes reflecting label specifications. After drying for approximately 24 hours indoors, groups of slabs were subjected to simulated rainfall events of 1 inch/hour for 1 hour using an indoor rainfall simulator designed to generate droplets of realistic sizes and energy and to provide uniform rainfall intensity across an area of around 8 by 15 ft. The slabs were held at an angle of 5 degrees to ensure the washoff flowed off the slab surface into collectors and ultimately into bottles that collected all the washoff. Three replicate slabs were inundated for each product; thus, along with control slabs, this generated fifty-seven individual washoff samples. Chemical analyses quantified the mass of the active ingredients found in the washoff and the data were compared in terms of the percentage of applied chemical that was removed by the simulated rainstorm. There was generally close agreement between the washoff percentages for replicate slabs for a given product indicating that this experimental design was suitable for conducting a comparative study of this type. Results will be compared and contrasted in the context of the behavior of general formulation categories and options for additional related research will be discussed.

**726 Surface Water Contamination Associated with Fipronil Use in Residential Landscapes** J. Wu, UF/IFAS-IRREC; J. Lu, Y. Lin, Univ of Florida/IFAS-IRREC, Soil and Water Science; P. Wilson, Univ of Florida, IFAS, IRREC- Soil & Water Science. The phenylpyrazole pesticide fipronil has been increasingly used for pest control in residential landscapes. The presence of fipronil and some of its' degradates in surface water has attracted public attention due to their potential risks to ecosystem and human health. A monitoring study was carried out from September 2008 to July 2010 to investigate the occurrence of fipronil and two of its metabolites in surface water associated with residential land-uses. Five canals and three stormwater retention ponds collecting surface water from residential land-uses were sampled and analyzed by GC-ECD weekly. Fipronil and fipronil sulfone were detected at five of eight sampling sites, with concentrations ranging from 0.5-210.6 and 0.5-77.1 ng L<sup>-1</sup>, respectively. The same respective compounds were detected in 3.1% and 1.0-5.2% of the samples collected from three of the canals, and in 33-45% and 78-81% of samples collected in two of the ponds. Except for the two retention ponds, fipronil and its metabolites were detected in less than 5% (most below 3%) of the samples collected. Fipronil sulfide was detected in 16-46% of the samples from the two ponds and in 1-2% from the other sites, at concentrations ranging from 0.4-26.9 ng/L. Contamination appears to be significantly influenced by irrigation management, as well as by rainfall/stormwater runoff events.

**727 Evaluating the Potential Ecological Impact of Perfluorinated Chemicals (PFCs) in Minnesota Lakes Using cDNA Microarrays** R.A. Lehr, Northland College, Natural Resources; R. Colli-Dula, Univ of Florida; C.J. Martyniuk, Univ of New Brunswick, Dept of Biology/Canadian Rivers Institute, Univ of New Brunswick, Biology; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. Perfluorinated chemicals (PFCs) stem from a wide range of sources and have been detected in stormwater runoff and aquatic ecosystems around the world. Laboratory research suggests that PFC exposure can result in a number of adverse health outcomes for aquatic organisms, but relatively little research has been conducted to assess whether PFCs are impacting the health of lakes and streams. To assess the potential impact of PFCs in aquatic ecosystems, gene expression patterns in largemouth bass were quantified across a PFC exposure gradient in lakes throughout the Twin Cities Metro Area (TCMA) in Minnesota using a 15,000 gene cDNA microarray. Gene expression patterns in largemouth bass corresponded closely with PFC concentration across the TCMA lakes, where fish from lakes with the highest PFC exposure were observed to have 5437 and 5936 differentially expressed genes in liver and gonad tissues, relative to reference sites. Similarly, largemouth bass with high PFC tissue concentrations consistently exhibited changes in the expression of genes related to lipid metabolism, energy production, RNA processing, protein production/degradation and contaminant detoxification—all of which are consistent with biomarker and bioindicator responses observed in previous laboratory studies with PFCs. Interestingly, although the biological process that are differentially regulated in largemouth bass with high PFC tissue concentrations are consistent with laboratory observations, the mechanisms of action associated with these changes are variable across studies. Taken together, these results suggest that

PFCs are potentially influencing the physiological condition of largemouth bass in several lakes throughout the TCMA. However, given the wide range of genes that are differentially expressed across the lakes and the variability observed in the mechanisms through which different biological processes have been affected, it is unlikely that PFCs are the only stressors affecting largemouth bass in TCMA lakes.

**728 Impact of CSO and Stormwater Runoff on Water Quality in Urban Streams** J. Bushey, Univ of Connecticut, Asst Professor; A. Aragon-Jose, Univ of Connecticut; C. Perkins, Univ of Connecticut, Center for Environmental Sciences and Engineering; M. Mendes, Univ of Connecticut; G. Ulatowski, Univ of Connecticut, Center for Environmental Sciences and Engineering. Combined sewer systems (CSSs) impact water quality of receiving water bodies, especially during high discharge events, in many urban systems. Such urban systems not only receive contaminant loading from stormwater, but also as a result of sewage contributions from combined sewer overflows (CSOs). A wide range of contaminants have been detected including nitrogen, phosphorous, trace metals, coliforms and anthropogenic organics. However, the chemical composition of stormwater and CSOs varies within an event, representing contributions from multiple sources. Management of contaminant loading often ignores these temporal shifts in speciation as well as fate within the receiving water body, further compounding the difficult and challenging problem that many municipalities face of assessing ecological impacts. To assess potential changes in loading and chemical speciation we have collected stream water samples in the Park River sewershed (Hartford, CT) during base flow and events to assess potential for contaminant loading and mobilization. Three events have been collected to date. Trace metal, TSS and DOC concentrations increased with discharge. However, trace metal concentrations and flux values reflected the degree of urbanization and industry present in the watersheds. All samples contained low DOC with the majority of the flux occurring in the particulate phase. Dissolved transport with DOC, particularly for Hg, decreased with urbanization. The degree of urbanization also increased TN flux as well as the distribution among N chemical species, with urbanized systems increasing in the NO<sub>x</sub> fraction.

**729 Urban Stormwater-associated Prespawn Mortality of Coho in the Greater Seattle Region: Could Simple Dissolved Organic Carbon Quality Explain It All?** J.A. Colton, King County Dept of Natural Resources, Water and Land Resources Division, King Cty. Dept of Nat. Resources, Water and Land Resources Division; R. Jack, King County, Dept of Natural Resources and Parks; D. Lester, King County Dept of Natural Resources, Water and Land Resources Division. Prespawn mortality of coho salmon is an acute mortality phenomenon associated with urban stormwater runoff. Substantial prespawn mortality rates (up to 90% of females) have been observed in many urban streams in the Seattle metropolitan area. The exact cause of the acute mortality is unknown but has been posited as one or more metals, pesticides, or some other organic chemical or group. This presentation discusses the potential for an alternative causal factor: dissolved organic carbon (DOC) quality. King County conducted a study in 2009 designed to compare water chemistry changes at baseline flow and throughout a storm event, and metals gill tissue levels in prespawn and successful-spawned coho between two streams: Longfellow Creek, a well-studied, high prespawn stream, and Lund's Gulch Creek, a reference stream. This study demonstrated dramatic changes in water chemistry (e.g., calcium, sodium, magnesium, copper, zinc) during storm events within a stream, the most extreme of which occurred in Longfellow Creek. Although metals concentrations in water peaked during a storm event, levels did not go above Washington State Water Quality Standards. However, lead accumulation in gill tissues tested as the only metal statistically higher in Longfellow Creek prespawn coho than Lund's Gulch coho. Although a hypothesis of hypocalcemia is entertained due to the combination of calcium drops, metals spikes, and weak calcium pumps in transitional coho, the potential for large differences in DOC quality became a strong option as well. King County conducted a second study in 2010 which measured UV absorbance as an index for DOC quality during baseline and storm flow in the same streams (as well as metals and cations). Published evidence is mounting for the importance of DOC quality in ameliorating metals toxicity and its role appears to be more diverse than previously thought (e.g., affecting gill physiology and metals bioavailability). The habitat conditions at each study stream are congruent with humic acids providing higher DOC quality and, thus, greater amelioration, at the reference stream. The 2010 UV absorbance results and water

chemistry data from both King County studies will be discussed in regard to their support for the DOC quality hypothesis.

**730 Stormwater Toxicity Challenges and Innovative Solutions – Case Study at UCSD's Scripps Institution of Oceanography** C. Stransky, AMEC Earth and Environmental, AMEC Earth & Environmental, Aquatic Sciences; A. Cibor, Nautilus Environmental, 4132 Avati Dr; G. Rosen, SPAWAR Systems Center Pacific, Environmental, SPAWAR Systems Center Pacific, Scientist; M. Colvin, AMEC Earth & Environmental, 4132 Avati Dr; K. O'Connell, Univ of California San Diego, EH&S Environmental Affairs, 4132 Avati Dr. The variable nature of stormwater runoff presents unique challenges with regard to accurately characterizing water quality and potential receiving water impacts. Assessment of stormwater toxicity is one of many measurements required in a variety of National Pollution Discharge Elimination System (NPDES) Permits. Toxicity testing provides a valuable measure of stormwater quality as it is a direct measure of potential effects on local biota. Toxicity also integrates effects due to multiple contaminants and site-specific water quality characteristics. Results of toxicity tests, however, are dependent on a number of factors that need to be carefully considered (i.e., species chosen, organism source and quality, biological endpoints measured, exposure duration, natural confounding factors, and test methods). Standard EPA whole effluent toxicity (WET) test methods were developed for continuous point source wastewater discharges and were not designed to assess the extremely dynamic and transient nature of non-point source stormwater runoff. The consequence for current stormwater monitoring programs is a reliance on unrealistic and disjointed lines of evidence, potentially resulting in inappropriate management decisions. For more realistic exposure methods to gain acceptance, there is a need to improve and standardize quality controls, and to simplify field application. Several methods will be highlighted including both modified sampling techniques and in situ testing. Results from a pilot study using new technology (the SEA-Ring) during dry and wet weather off the Univ of California's Scripps Institution of Oceanography in 2010 and 2011 will be presented alongside concurrent routine NPDES monitoring data required for their discharges to an Area of Special Biological Significance. The prototype SEA-Ring consists of a circular carousel capable of housing an array of toxicity chambers and passive sampling devices that can be used to assess potential impacts due to a variety of contaminants and exposure pathways in situ.

**731 Defining Nano: An Investigation of the Size-dependent Effects of Nanoparticles** K. Sellers, ARCADIS, Principal Environmental Engineer; M. Bartee, C. Hassinger, ARCADIS. Efforts to characterize the toxicity of nanomaterials implicitly incorporate a definition of what it means to be "nano". Members of the scientific community and regulators around the world commonly use a definition of 1 to 100 nanometers (nm) in size. But uncertainties about this definition persist, with some defining nanoparticles as sizes up to 2000 nm and others proposing definitions that incorporate size ranges or surface area metrics. A preliminary examination of the literature presented in a poster at SETAC 2010 found that many tests did show a size-dependent effect. Most tests showed that decreasing particle size increased the effects of exposure to the test substance. However, the results suggested that – logically enough – decreasing the particle size and thereby increasing the surface area to volume ratio may not markedly increase the bioavailability of very poorly soluble materials. In this presentation, the authors will discuss the results of a meta-study of the ecotoxicology literature that examines the size-related behavior of particulates. The objective of this work is to determine whether the data show one or more "bright lines" that suggest specific relationships between certain particle sizes or other particle characteristics and the potential for specific behaviors or effects. The research will focus on a nanoparticle that is well-studied and has relatively poor water solubility, thus eliminating the ion toxicity variable (i.e., dissolved concentrations versus nanoparticle toxicity). This meta-study will provide an ecotoxicology view of the ongoing debates about how to define nanomaterials for the purpose of regulatory toxicology.

**732 Dynamic Measurement of Size and Fractal Structure of Single-Walled Carbon Nanotubes: Role of Chirality** N.B. Salch, I.A. Khan, Univ of South Carolina, Civil and Environmental Engineering; P.L. Ferguson, Duke Univ; T. Sabo-Attwood, Univ of Florida, Dept of Environmental & Global Health. Single-walled carbon nanotubes (SWNTs) have widespread application due to their unique physicochemical properties. To-date, there has been no studies addressing the effects of SWNT electronic structure,

i.e., chirality/helicity, on its aggregation behavior in biological and natural systems. This study is the first of its kind to address aggregation kinetics and fractal dimension of chirally separated SWNTs in chemistries relevant to natural aqueous systems and biologically relevant conditions. SWNTs have been chirally separated using density gradient ultracentrifugation and analyzed using near-IR fluorescence spectroscopy. Kataura plots show a narrow distribution of chirally separated SWNTs. Detailed physicochemical and electrokinetic characterization are performed using Raman Spectroscopy, thermal gravimetric analysis, electron microscopy, and electrophoretic mobility measurements. Fundamental aggregation kinetics study with specific, i.e., (6,5) and (7,6), and mixed chiralities is performed using time-resolved dynamic light scattering. Angle-dependent static light scattering (SLS) technique is employed to measure  $D_f$  of carboxylated SWNTs of the aforementioned chiralities under natural and biologically relevant conditions. Aggregation of SWNTs followed typical DLVO type behavior. Kinetics of SWNTs varied significantly with the change in chirality and dominated by van der Waals interaction as a function of chirality. The SLS-intensity data are collected for the entire scattering range (Guinier through Fractal to Porod regimes, i.e., 12-150°) and results demonstrate strong effect of chirality and weak dependence of biological media conditions on  $D_f$ . Aggregation kinetics data and  $D_f$  values thus obtained can provide with unique insight on a more realistic SWNT exposure and fate in biological and natural environments.

**733 Assessing the Environmental Risks of Nanomaterials: Critical Review of Risk Analysis Frameworks** K. Grieger, Technical Univ of Denmark, Dept of Management Engineering; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center; S. Hansen, Technical Univ of Denmark; A. Baun, Technical Univ of Denmark, Dept of Environmental Engineering. Risk assessment frameworks and strategies for engineered nanomaterials (NM) are currently being reviewed and adapted in many cases. Thus far, this has proved to be a challenging task, as well as likely extremely time- and resource-consuming. In response to these grand challenges for NM, a number of alternative or complimentary frameworks have been proposed in recent years to standard risk assessment. However, further information on these alternative approaches such as their potential strengths and limitations is currently unavailable. This analysis therefore evaluates these different risk analysis frameworks which have been proposed for NM based on a number of select criteria and specifically focusing on environmental risk analysis for NM. The following criteria were used for this evaluation: flexible for a variety of NM, suitable for multiple decision contexts, incorporate uncertainty analysis, include life cycle perspectives, iterative or adaptive, enable more timely decision making, transparent, integrate various stakeholder perspectives, integrate precaution, and include qualitative or quantitative data. Results showed that most of the investigated frameworks were i) flexible for multiple NM, ii) suitable for multiple decision contexts, iii) included life cycle perspectives, iv) transparent, v) included precautionary aspects, and vi) able to include qualitative and quantitative data. It was also found that many of the frameworks may be adapted for iterative or adaptive elements and timely decision making if needed, although these were not inherently embedded in many of the frameworks based on their current formats. In addition, most frameworks were mainly applicable to occupational settings with minor applications for the environment, and many if not most of the frameworks had not been thoroughly tested on a wide range of NM or nano-applications. Given these results, we recommend using a multi-faceted approach to assess the environmental risks of NM in which different frameworks may be used together and/or combined for a particular risk context. We also recommend additional testing of these frameworks on concrete NM applications which are specifically relevant for environmental risk contexts.

**734 Using Value of Information to Prioritize Nanomaterial Research** M. Barber, MIT & Army Corp of Engineers; M.E. Bates, US Army Corps of Engineers, Environmental Lab, Engineer Research and Development Center; J. Kiesler, Univ of Massachusetts; J.A. Steevens, US Army Engineer Research & Development Center, Waterways Experiment Station; M.A. Chappell, US Army Corps of Engineers, Environmental Laboratory, US Army Corps of Engineers; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center. Even though, in recent years, much research has been done on nanomaterials and their properties, there are still large gaps in our knowledge of nanomaterial risk. While there are many uncertainties in nanomaterial risk, particularly when it comes to the human health and

environmental toxicology, having more information about certain risks may be more valuable than having more information about other risks. As more and more nanomaterials are developed each year, researchers must continually prioritize on which nanomaterials to focus their research. Multi-Criteria Decision Analysis can be used to frame the decision problem and to quantify uncertainty in risk effects, yielding an initial prioritized list of research agendas. Value of Information (VoI) analysis can then be used to determine how, if given some additional information on the risk effects of a particular nanomaterial, that prioritized list would change. The combined MCDA/VoI framework can help scientists and decision-makers select more complete and more accurate lists of prioritized research agendas to investigate nanomaterial risks.

**735 Dissolution of Retained Silver Nanoparticles in Water-saturated Porous Media** Y. Wang, A. Taghavy, A. Mittelman, M.D. Becker, L.M. Abriola, Tufts Univ, Civil & Environmental Engineering; K. Pennell, Tufts Univ, Dept of Civil & Environmental Engineering. Concerns over potential health and environmental impacts of silver nanoparticles (nAg), which are widely used as biocidal agents, call for a thorough understanding of their fate in the environment. Coupled experimental and mathematical modeling studies were conducted to investigate mechanisms governing the transport, retention and dissolution of nAg in a water-saturated quartz sand as a function of pH and oxygen content (DO). For each experiment, a 3 pore volume (PV) pulse of 3 mg/L nanosilver suspension (stabilized with citrate, conditioned with 10 mM sodium nitrate or sodium acetate, and pH of 4 or 7) was introduced into a column (10cm length x2.5cm dia.) packed with 40-50 mesh Ottawa sand (dH = 355  $\mu$ m) at a pore-water velocity of ca. 7.6 m/d. At neutral pH concentrations of dissolved Ag<sup>+</sup> in the column effluent were low (<0.03 mg/L) and approximately 25% of the injected nAg mass was retained within the column. During the subsequent elution period (30 PVs of pH 4 particle-free solution), Ag<sup>+</sup> concentrations achieved a maximum value of 0.12 mg/L and no nAg were observed in the effluent for the entire period, indicating that retained nAg were slowly dissolving. Effluent Ag<sup>+</sup> concentrations increased slightly (up to 0.15 mg/L) at the higher DO (8.9 ppm), but decreased markedly (< 0.01 mg/L) when pH was increased to 7.0. A hybrid Eulerian-Lagrangian simulator was developed to describe the coupled reactive transport of nanoparticles and dissolved constituents. Implementation of an empirical expression for the nAg dissolution coefficient facilitated an accurate representation of the observed nanoparticle dissolution behavior. Simulation results demonstrate the relative insensitivity of nAg dissolution to DO. Model sensitivity analyses revealed that pH, residence time and nanoparticle specific surface area were the primary factors controlling nAg dissolution.

**736 A Case Study for the Release of Nano Particulate Aluminum from the Use of a Nanotechnology** J.A. Stevens, US Army Engineer Research & Development Center, Waterways Experiment Station; V. Boddu, A. Bednar, M. Chappell, C. Griggs, A. Kennedy, A. Poda, C. Weiss, US Army ERDC; V. Korampally, S. Gangopadhyay, Univ of Missouri; K. Gangopadhyay, NEMS/MEMS LLC; R. Thiruvengadathan, Univ of Missouri. Currently there are very few studies that have examined the release of particulates associated with a nano-based technology. Most studies examining the potential environmental risks of nanoparticles have focused on raw "as produced" nanoparticles that have not yet been incorporated into a technology such as a composite, coating, or other matrix. In this study, we examine the residue of nanometal-based energetics following ignition; the most likely release of nanoparticles from a specific nanotechnology. Metals such as aluminum and copper offer great potential for use as energetic such as explosives propellants, and primers. In this study, green primers were ignited to collect residual material. Aluminum particles of average particle size (APS) of 80 nm having active metal content of 80 percent by weight, and 2.2 nm oxide shell layer were used as fuel in the preparation of energetic mixtures. These materials were composed of a mixture of CuO nanorods and Al nanoparticles or CuO nanorods and Al micron flakes (75% by wt) combined with nitrocellulose (25% by wt). These materials represent residues expected after ignition as part of an energetic. Materials were examined pre and post ignition. Ignition resulted in a low production of residue for characterization (10-15 %). Broad analysis by SEM shows formation of a wide range of particulate sizes (0.01 to > 100  $\mu$ m) for the nano based energetic while the micron sized energetic is composed primarily of large (>10  $\mu$ m) aggregates. It is expected the smaller size of the nano energetic residue, when combined with the acidic conditions of an ignition will result in a higher dissolution

rate for aluminum and copper from the primer. Results of this study represent one of the first documented releases of a nano-sized particulate from a nano-enabled technology.

**737 Risk Quantification of Engineered Nanomaterials in Water Based on Probabilistic Species Sensitivity Distribution Modeling** B. Nowack, EMPA, Empa-Materials Science and Technology; E. Kost, F. Gottschalk, Empa-Materials Science and Technology. In this study we present a quantification of the risks of five engineered nanomaterials (ENM), TiO<sub>2</sub>, ZnO, Ag, CNT, fullerenes, to aquatic organisms by linking probability distributions of modeled environmental concentrations to species sensitivity data. We evaluated published ecotoxicity tests with aquatic organisms and derived for each species and study no effect concentrations (NEC) according to the ECHA guideline<sup>1</sup>, using assessment factors to transform acute to chronic, L(E)C<sub>50</sub> to chronic NOEC and NEC, and to account for laboratory-field variability. We then combined all data to derive probabilistic species sensitivity distributions (PSSD) that account for the large differences in effects of one ENM to the same organisms in different studies. Using this approach we therefore consider different forms, aggregation states, coatings, etc. of the ENM into the species sensitivity distribution. The modeled PSSD were then compared to modeled predicted environmental concentration (PEC), also in the form of probability distributions<sup>2</sup>. The results show that there is a 4.6% chance for a risk to organisms in surface water to nano-Ag and 0.008% for nano-ZnO. For the other three ENM no risk was predicted. In undiluted effluent from wastewater treatment plants (representing a worst-case scenario), the risks are 45% for nano-Ag, 35% for nano-ZnO and 62% for nano-TiO<sub>2</sub>. The risks for fullerenes and CNT are negligible. These results show that using realistic ranges of environmental concentrations, slight risks to aquatic organisms might be present for nano-Ag, nano-ZnO and nano-TiO<sub>2</sub>. These ENM should therefore be investigated further, especially with regards to their chronic toxicity and their actual form in the environment. Literature 1. ECHA, Guidance on information requirements and chemical safety assessment Chapter R.16: Environmental Exposure Estimation, European Chemicals Agency. 2010. 2. Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B., Modeled environmental concentrations of engineered nanomaterials (TiO<sub>2</sub>, ZnO, Ag, CNT, fullerenes) for different regions. *Environ. Sci. Technol.* 2009, 43, 9216-9222.

**738 Differential Uptake Between Ionic Cadmium and Quantum Dot Released Cadmium** B.C. Seda, Clemson Univ, Graduate Program of Environmental Toxicology, Clemson Univ; A.S. Mount, Clemson Univ, Biological Sciences; A. Roberts, Univ of North Texas; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology (ENTOX) Much of the toxicity of semiconductor quantum dots is attributed to the release of heavy metal ions, such as cadmium in the case of CdSe/ZnS quantum dots. Because of the unique optical properties of quantum dots, their uptake in small transparent organisms such as *Daphnia magna* can be observed by fluorescence microscopy techniques. In *D. magna* that have been exposed to surface modified CdSe/ZnS quantum dots, fluorescence indicates the quantum dots accumulate in the gut tract, where they can remain for more than 24 hours, providing for circumstances allowing the gradual release of Cd. However, the amount of Cd retained in the whole organism after this time varies with particle surface chemistry. In this study, *D. magna* were exposed to CdSe/ZnS quantum dots and the equivalent concentration of Cd. Confocal fluorescence laser scanning microscopy (LSM) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) techniques were used to detect uptake of intact quantum dots and cadmium, respectively. Because polyethylene glycol coated CdSe/ZnS quantum dots were previously observed to not cross gut epithelium, they were not expected to do so in this study. LA-ICP-MS was used to detect metals concentrations in tissues not associated with the gut tract or external tissues. Our hypothesis is that Cd released from the quantum dots will be observed to have been absorbed by the *D. magna*. Because evidence suggests that the quantum dots are associated with the gut tract, even after depuration, tissue specific Cd concentrations were expected to be similar between ionic Cd and quantum dot exposures.

**739 Applying Ecosystem Services to the Ambient Air Quality Setting Process** A.W. Rea, US Environmental Protection Agency, Office of Research and Development. The US Environmental Protection Agency (EPA) currently is conducting a joint review of the existing secondary



(welfare-based) National Ambient Air Quality Standards (NAAQS) for oxides of nitrogen (NO<sub>x</sub>) and sulfur (SO<sub>x</sub>). EPA has decided to assess jointly the science, risks, and policies relevant to protecting public welfare associated with oxides of nitrogen and sulfur due to both their atmospheric interactions and ecological effects. As stated in the Clean Air Act (CAA), the purpose of a secondary NAAQS is to "protect the public welfare from any known or anticipated adverse effects associated with the presence of such air pollutants in the ambient air." Assessments of adverse public welfare effects are based on how ecologically adverse impacts translate into adverse impacts on public welfare via changes in ecosystem service delivery. While adversity is not explicitly defined in the CAA, it can be inferred that adverse ecological impacts have some corresponding impact on the well-being of human populations, through reductions in ecosystem services that might include direct (e.g., recreational fishing) or indirect (e.g., provision of habitat for endangered species) services to humans. These ecosystem service linkages are being used to inform the standard setting process. The ecological risk assessment focused on several case studies in sensitive ecosystems. Ecosystem services were used to characterize adversity to public welfare of ecological effects associated with current levels of nitrogen and sulfur deposition. For aquatic acidification, ambient concentrations of NO<sub>x</sub> and SO<sub>x</sub> were linked to deposition using a critical loads approach, projecting changes in an ecological indicator (acid neutralizing capacity, ANC) correlated with changes in fish species richness and its influence on recreational fishing. For terrestrial acidification, a critical loads approach was used to link base cation:aluminum ratios to tree health and associated provision of wood products (sugar maple production and timber production). For nitrogen enrichment in estuaries, a eutrophication index was linked to degradation, hypoxia, and diversity loss associated with changes in recreational fishing and shifts in plant species composition, and with nitrogen cycling to benefits received from hunting, fishing, and wildlife viewing. An update on the current status of the review and a summary of the proposed rulemaking (July 2011) will be provided.

**740 Communicating Ecosystem Services: Tools for Scientists to Engage the Public** C.S. Duke, Ecological Society of America. The Ecological Society of America (ESA), in partnership with the Union of Concerned Scientists, developed the Communicating Ecosystem Services project with the goal to increase the public's awareness of the importance of ecosystem services. The project provides series of Tool Kits for scientists, educators, and other professionals to help them address your local media, community groups, state legislators or other audiences. The Tool Kits provide background information on specific ecosystem services, as well as general tips and suggestions for developing presentations or writing articles for a non-scientific audience. Tool Kits have been completed for pollination, water purification, and marine nurseries ecosystem services. Each consists of the following elements: basic fact sheet, what you can do to preserve the service, key points, science summary, resources and references, case studies, policy overview, and presentation aids. All the information is available without charge at [www.esa.org/ecoservices](http://www.esa.org/ecoservices). This presentation will describe the tool kits and summarize their contents.

**741 Ecosystem Services as Assessment Endpoints in Ecological Risk Assessment** W.R. Munns, US. EPA, Atlantic Ecology Division; A.W. Rea, USEPA, National Exposure Research Laboratory; M.G. Barron, USEPA, Gulf Ecology Division; S. Jordan, USEPA. The focus of ecological risk assessment (ERA) is on assessment endpoints, explicit expressions of environmental values to be protected. Traditionally, the ecological entities identified in assessment endpoints have been components of ecosystems deemed by risk assessors to be important to ecosystem structure and function – the benthic community, for example. Yet, there is growing awareness that improved environmental management can be achieved by considering more explicitly how decisions affect the well being of people and society. Ecosystem services, the outputs of ecological functions or processes that contribute to social welfare, can complement traditional assessment endpoints by clarifying to the public the benefits and costs a decision will have to society. Ecosystem service assessment endpoints can improve the transparency of decision making by focusing ERAs on components of nature that nonscientists understand and value. By enabling a more complete evaluation of the tradeoffs involved with the alternative solutions being considered, decision makers can be more fully informed about the intended and possible unintended consequences of their choices. This presentation will describe efforts currently underway by the USEPA's Risk Assessment Forum to establish the technical foundation needed to incorporate ecosystem services in ERA,

and to identify generic ecosystem service assessment endpoints that can be considered during problem formulation. Several candidate generic ecosystem service assessment endpoints are considered, including catchable fish, clean water and air, climate regulation, water supply and flood protection, arable land and agricultural productivity, and aesthetic values. General guidance is being developed for selecting ecosystem service assessment endpoints, and for translating risk to traditional assessment endpoints to risk to ecosystem services. Several case studies are being conducted to highlight how use of ecosystem service assessment endpoints can enhance decision making that span several spatial scales and types of decisions. These include case studies covering local issues involving hazardous waste sites and conductivity in Appalachian streams, to broader issues encompassing threatened and endangered species and national scale assessments for setting air quality standards.

**742 Examples of Ecological Services Accounting Using Habitat Equivalency Analysis in Support of Environmental Decision Making** R. Gouguet, Windward Environmental LLC. Habitat equivalency analysis (HEA) was originally developed by the National Oceanic and Atmospheric Administration as a natural resource damage assessment tool for quantifying natural resource service losses and calculating the scale of compensatory restoration required to offset those service losses. HEA can also be used to calculate and track service flow changes in areas such as mitigation requirements development, quantitative dredge material management alternative analysis, remedial alternative analysis, and ecological compensation calculation in lieu of active cleanup. The general approach includes estimating baseline conditions and gains or losses in ecological services associated with various actions based on the judgment of professionals with knowledge of the local ecosystem. Estimated changes in ecosystem service levels under projected post-action conditions are summed over time, allowing a comparison of the net present ecological service value under each scenario and the selection of the "most appropriate" alternative. This presentation will describe how ecological services accounting was used as a planning tool at five sites: (1) Craney Island, Virginia; (2) Matagorda Bay, Texas; (3) Swan Lake, Texas; (4) Port Arthur, Texas; and (5) Woodard Bay Natural Resources Conservation Area, Washington.

**743 The Role of Ecosystem Services as a Management Tool for Protecting Marine Preserves in Southern California** S. Gruber, Weston Solutions, Inc., Weston Solutions; M. Wartian, Weston Solutions, Inc, Water Resource Services; D.W. Moore, Weston Solutions, Inc, Director Natural Resource Management; B.J. Mastin, Weston Solutions, Inc., Natural Resources. Along the southern California coast, marine preserves known as Areas of Special Biological Significance (ASBS) have been established through the California Ocean Plan to protect the beneficial uses of these extremely valuable coastal resources. Due to their close proximity to urbanized watersheds in the coastal zone, compliance monitoring programs known as the ASBS Special Protections have been established by the California State Water Resources Control Board (State Board) to protect the beneficial uses from urban runoff and assure that water quality in the ASBS does not differ from natural water quality found at un-urbanized reference sites. Although there are some monitoring elements in the Special Protections that assess toxicological and biological endpoints, the primary focus of the Special Protections is on water quality during storm events. However, recent studies conducted by Weston Solutions at several ASBS in southern California suggest that impacts from storm water runoff may be ephemeral and have limited impacts to the resources in the ASBS receiving waters relative to other perturbations, such as long-shore transport of contaminants from nearby harbors and trampling of intertidal biota by anglers, tide pool visitors, and school groups. In order to truly protect and enhance the beneficial uses of the ASBS, the ecosystem services concept is being applied in implementing a monitoring and restoration strategy for several ASBS in the region. The program focuses on a comprehensive approach to understanding all the potential impacts to ASBS resources, minimizing those impacts through a suite of prioritized best management practices, while enhancing the recreational services provided by the ASBS for anglers, tide pool visitors, and educators. This comprehensive approach using the ecosystem services concept has important implications for future management of these extremely valuable coastal preserves.

**744 The Use of Ecosystem Services Information by the National Estuary Program** L. Martin, US Environmental Protection Agency, Risk Assessment Forum; I. Purdy, US Environmental Protection Agency, Region 2

Watershed Management Branch. Ecosystem Services concepts and data are rapidly evolving through their use and application. This development is in part motivated by the belief that incorporation ecosystem services information (monetary or non-monetary valuation) will improve environmental decision-making and outcomes in those projects whose management decisions are informed by risk assessment and some form of a cost-benefit analysis. This presentation will report on a three-year exploration of the National Estuary Programs (NEP) use of ecosystem services concepts, and outcomes in meeting management objectives. The study examined attitudes toward and experience with incorporation of ecosystem services in environmental management programs for a self-selecting subset of the 28 member NEP. The study included use of targeted questions, workshop presentations and scheduled conference call dialogs between EPA and the NEP staff and managers. Results indicate that the willingness to focus on ecosystem service concepts by the NEP programs' environmental managers correlate well with the ability to use them within the existing management and accountability paradigm.

**745 Economic Valuation Tools and Their Application to Ecosystem Services** M. Mazzotta, EcoBenefits Research. Many of the ecosystem services that people value are not normally bought and sold; yet, this does not mean they have no measurable economic value. Economists have developed a set of approaches and tools for valuing the full range of both market and "non-market" ecosystem goods and services. These approaches and tools offer a way to understand and evaluate the many tradeoffs involved in managing natural resources and environmental risks, to answer questions such as: How can we allocate scarce resources to provide the greatest benefits to society?; or How much additional risk are people willing to accept in exchange for other valued goods and services? While value is inherently subjective, economic valuation methods aim to quantify it by looking at the trade-offs people are willing to make, which can reveal their values for different states of the world. An overview of economic valuation tools for ecosystem services, focusing on non-market valuation, will be presented, along with some basic economic concepts that are important to economic valuation. These concepts include: the difference between economic impacts and social benefits; marginal versus total value; and dollar values versus non-monetary values and when each is most appropriate. While economic valuation tools can be tremendously useful, there are often practical limitations to their application, and they provide only one form of information to be used in conjunction with other tools and approaches to inform decisions. Thus, some caveats will be presented, and economic valuation approaches will be discussed in the context of the frameworks where they are typically applied.

**746 Ecosystem Services in Soil Policy: An Example** J. Van Wensem, Soil Protection Technical Committee (TCB), TCB. In many countries the issue of chemical contamination of soils is traditionally an important part of the policy. This has resulted in a strong focus on standard setting -, risk assessment- and remediation methods, thereby often neglecting other aspects of good soil quality. In the past ten years biological, physical, and ecological aspects, land use, fitness for use and ecosystem services have gained more attention from policy makers. The recognition that good soil quality is not only determined by the chemical composition of the soil is the driver behind this development. The concept of ecosystem services is slowly infiltrating in Dutch soil policy. First it was recognized that soil quality, besides chemical, has also physical and biological aspects. More focus was given to land use and the necessary soil quality to support specific types of land use. Next, a method was developed to measure biological qualities given the land use, also allowing to express these qualities in terms of the performance of – mostly – supporting ecosystem services. Recently, pilot projects have started to help municipalities to look at the soil in terms of ecosystem services and tradeoffs, thereby connecting local soil policy with spatial planning and societal demands. Early 2011 the Dutch Soil Protection Committee has been asked to prepare a recommendation for the government on how to facilitate optimal use of ecosystem services by land users and local authorities. These developments are paralleled by the European soil strategy. It is too early to predict the final result of these processes, but promising steps have been made. The presentation will focus on the steps that have led to the introduction of more integrated approaches, based on the ecosystem services concept.

**747 What are Forensic Approaches to Ecotoxicology?** J.E. Elliott, Environment Canada, Pacific Wildlife Research Centre, Science and Technology

Branch, Environment Canada, Pacific Wildlife Research Centre, Environment Canada, Canadian Wildlife Service. This introductory talk provides an overview of the session, and therefore of the book, and some discussion of the background and of emergent themes. The nature of forensic ecotoxicology is considered, and a definition proposed. I reflect on the experiences of some authors in trying to establish cause-effect, and the challenges of translating scientific evidence of toxicant effects into regulatory or non-regulatory action. I further examine the problem of bias and some ethical aspects in data interpretation, and consider some of the common dispute resolution processes encountered and discussed by the various authors.

**748 Toxic Trees: Arsenic Pesticides, Woodpeckers and the Mountain Pine Beetle** C. Morrissey, Univ of Saskatchewan, Biology; J.E. Elliott, Environment Canada, Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada, Pacific Wildlife Research Centre, Environment Canada, Canadian Wildlife Service. During the mid-1990s, an unprecedented outbreak of the Mountain Pine Beetle (*Dendroctonus ponderosae*) in the economically valuable pine forests of British Columbia triggered a major campaign by forest managers to try and control the beetle damage. The result was a major change in the landscape from harvesting and prescribed burns in addition to the wide scale application of a systemic insecticide known as monosodium methanearsonate (MSMA). Since MSMA contains arsenic, is highly stable, and was being applied in increasingly large quantities, our study evaluated the potential impacts to forest birds, particularly woodpeckers. From 2002-2006, we investigated the exposure of breeding woodpeckers to MSMA ingested via contaminated bark beetles. We measured high levels of arsenic in beetles from treated trees, and found significant amounts of debarking on many MSMA-treated trees indicating woodpeckers were feeding on them. Radio-telemetry confirmed that woodpeckers breeding near MSMA treatments regularly used those stands. Blood samples of woodpeckers and other forest birds revealed elevated arsenic concentrations. Through a concurrent laboratory dosing study of Zebra finches (*Taeniopygia guttata*), we estimated woodpeckers were receiving enough MSMA through ingestion of contaminated beetles to cause poorer growth and mortality of young birds and mass loss in adults. We concluded that the combination of extensive harvesting in the region in combination with the large numbers of treated MSMA trees was potentially detrimental to forest bird populations. By the end of our study, MSMA approval for use in Canada was revoked and the Ministry of Forests did not pursue re-registration of MSMA. We clearly demonstrated that large scale MSMA use had the potential to cause serious harm to forest birds, while appearing to have limited efficacy in beetle control. This was a landmark study in the field of wildlife ecotoxicology – a first to evaluate the exposure and effects of a toxic chemical to woodpeckers in forest ecosystems.

**749 Selenium, Salty Water, and Deformed Birds** H. Ohlendorf, CH2M Hill. Selenium was identified in the 1930s as the cause of embryo mortality and severe embryo deformities when chickens were fed grains grown on seleniferous soils in South Dakota. There had been no documented occurrences of such effects in wild birds before 1983, when we studied the effects of agricultural irrigation drainage water contaminants on birds feeding and nesting at Kesterson Reservoir, located within the Kesterson National Wildlife Refuge in the San Joaquin Valley of California. The Reservoir was used for disposal of subsurface saline drainage waters from agricultural fields and was intended to provide beneficial habitat for wildlife, particularly waterfowl and other aquatic birds. Analyses of food-chain biota (plants, aquatic invertebrates, and fish) and bird tissues or eggs showed that selenium was the only chemical found at concentrations high enough to cause the observed adverse effects on bird health or reproduction. Results of the studies at Kesterson Reservoir stimulated interest and concern about the effects of selenium in agricultural drainage throughout the western USA where similar scenarios might exist (as well as in industrial settings such as mining and power generation). Those studies showed that selenium-related problems with agricultural drainage were widespread and locally significant. Problems of managing selenium in agricultural drainwater are difficult to solve or mitigate, despite intensive efforts to do so. This presentation briefly describes the field and laboratory studies that documented the effects of selenium in birds using wetlands receiving seleniferous agricultural drainage, the linkages between those studies and subsequent efforts to address the issue of selenium contamination in agricultural drainage water, and the consequent conservation gains.

**750 Rocky Mountain Arsenal: Chemical Weapons, Cyclodiene Pesticides, and the Making of an Urban Wildlife Refuge** J.T. Edson, Edson Ecosystems LLC; J.V. Holmes, Stratus Consulting Inc.; J.E. Elliott, Environment Canada, Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada, Pacific Wildlife Research Centre, Environment Canada, Canadian Wildlife Service; C. Bishop, Environment Canada, Science And Technology Branch, Environment Canada, Canadian Wildlife Service. In 1942, the US Army purchased 70 km<sup>2</sup> near Denver, Colorado, to construct the Rocky Mountain Arsenal, where they manufactured chemical and incendiary weapons (mustard, lewisite, napalm) in support of the war effort. After World War II, Shell Oil and its predecessors manufactured pesticides, insecticides, and herbicides at the Arsenal, and the Army manufactured and then decommissioned Sarin (GB) nerve agent. The industrial manufacturing was concentrated near the center of the site, with many square kilometers of undeveloped land providing a buffer from urban Denver. Millions of liters of liquid wastes, including cyclodiene pesticides such as dieldrin, aldrin, and endrin, were disposed of in open basins, pits, and trenches on the site. Cyclodiene pesticides impact the central nervous system of exposed biota, causing disorientation, emaciation, and eventually death. Thousands of wildlife mortalities were documented at the site, including an estimate of 20,000 duck deaths over a 10-year period in the 1950s, and over 1,800 waterfowl deaths in one basin alone between 1981 and 1987. After manufacturing ceased in the 1980s, the Army, Shell, the US Environmental Protection Agency, and the State of Colorado endeavored to address the contamination and the Arsenal's future land use. In 1992, the USA passed the Rocky Mountain Arsenal National Wildlife Refuge Act to create wildlife habitat from uncontaminated and remediated areas and reduce risk of exposure to humans. Many aspects of the cleanup were based on pesticide risks to wildlife; however, the parties rarely reached agreement on appropriate cleanup thresholds and the size of the area requiring cleanup. Ultimately, in 2010, after nearly 30 years of investigation and cleanup efforts costing over \$2 billion, "significant environmental cleanup" was completed. Millions of tons of toxic sludges and soils had been placed in hazardous waste landfills, and millions of tons more were buried in place and capped. The refuge now comprises approximately 6,000 ha, roughly the size of Manhattan. The cleanup process was contentious, resulting in multiple lawsuits, which is not a model that the authors recommend. Although contamination in some parts of the Arsenal likely is still present and should continue to be evaluated, the refuge will be an important protected oasis of wildlife habitat in the midst of the Denver urban sprawl.

**751 Restoration of Lake Apopka's North Shore Marsh: High Hopes, Tough Times, and Persistent Progress** R.H. Rauschenberger, US Fish and Wildlife Service; R. Conrow, E.F. Lowe, M.F. Coveney, St. Johns River Water Management District; G. Masson, US Fish & Wildlife Service. The story of Lake Apopka is a familiar one to many Floridians and has gained international notoriety. The 12,500 ha lake was once a world-class bass fishery. Then, a century-long decline occurred, traced to the loss of over 8,000 ha of wetlands to farming operations, agricultural discharges laden with phosphorus to the lake, treated wastewater discharges, and input from citrus processing plants. The state of Florida and the Federal Government purchased the 8,000 ha property with the goal of restoring wetland habitat to drastically reduce nutrient-loading and thereby restore the lake. Shortly after flooding the property in the winter of 1998-1999, a bird mortality event occurred, resulting in the deaths of 676 birds, primarily American white pelicans (*Pelecanus erythrorhynchos*), but also including 43 endangered wood storks (*Mycteria americana*), 58 great blue herons (*Ardea herodias*), and 34 great egrets (*Casmerodius albus*). The deaths of the birds, attributed to pesticide toxicosis, was followed by years of research and remediation to ensure the future safety of wildlife on the property. Presently, about 3,000 ha of wetlands have been rehydrated since resuming restoration activities, with no adverse effects to wildlife. We present and discuss the history of Lake Apopka, the efforts to restore it, and what we have learned along the way.

**752 Abnormal Alligators and Organochlorine Pesticides in Lake Apopka, Florida** R.H. Rauschenberger, US Fish and Wildlife Service; A.R. Woodward, Florida Fish and Wildlife Conservation Commission; H.F. Percival, US Geological Survey, Florida Cooperative Fish and Wildlife Research Unit; T. Gross, Environmental Resource Consulting, Dr. Timothy S. Gross, Univ of Florida; K.G. Rice, US Geological Survey, Southeast Ecological Science Center; R. Conrow, St. Johns River Water Management District. Lake Apopka is a 12,400-ha hypereutrophic lake in central Florida

that was the recipient of nutrient and pesticide pollution from adjacent agricultural operations and treated wastewater for 50 years. The abnormal American alligators (*Alligator mississippiensis*) in Lake Apopka have been the object of a number of studies including investigations of a population crash, the epidemiology of egg failure, and anomalous endocrine function. Several hypotheses of the causes of these abnormalities have been proposed and examined by multiple research organizations over the past three decades. Initially, organochlorine pesticide (OCP) contamination was considered the factor most likely contributing to poor reproductive success. Concentrations of DDE in alligator eggs sampled in 1984-85 were approximately 4 mg/kg and those of toxaphene were approximately 2.5 mg/kg. These levels were known to cause reproductive failure in certain birds. However, transmissible diseases, population age and density, cyanotoxins, nutritional deficiencies, and combinations thereof, were also investigated for their contribution to poor alligator reproductive success. Investigations of an alligator mortality and reproductive failure event on Lake Griffin, a lake similar to Lake Apopka but with lower OCP levels, revealed analogous reproductive abnormalities that were associated with a dietary thiamine deficiency. Thiamine deficiency appeared to be associated with a diet of almost exclusively gizzard shad, which contain thiaminase, an enzyme that breaks down thiamine. OCP contaminants may contribute to these maladies, perhaps through endocrine disruption and increased stress. We present and discuss the findings of the past 30 years of work at Lake Apopka and their influence on local management decisions as well as policy at the national level.

**753 South Asian Vultures in Crisis: Environmental Contamination with a Pharmaceutical** L. Oaks, Washington State Univ, College of Veterinary Medicine; R.T. Watson, Peregrine Fund. In the late 1990s an unprecedented decline in the population of two of the world's most abundant raptors, the Oriental White-backed vulture (*Gyps bengalensis*) and the Long-billed vulture (*Gyps indicus*), was noticed in India. By the early 2000s, similar catastrophic declines followed in neighboring Pakistan. Ecological and forensic studies ultimately found that a non-steroidal anti-inflammatory pharmaceutical, diclofenac, was responsible. Diclofenac, long used in human medicine, had found its way into the veterinary market as a safe, inexpensive, and very popular drug for livestock in Southern Asia. Unfortunately, diclofenac residues caused kidney failure in *Gyps* vultures that fed on treated carcasses. And the loss of breeding adult vultures had a profound impact on the population, leading to declines on the order of 30% per year. In 2004, a series of meetings were held with government officials to inform them of this discovery. The extensive lobbying efforts that followed successfully led in 2006 to a ban on the manufacture of veterinary diclofenac in India, Pakistan, and Nepal. Sadly, in 2010, diclofenac still appears to be readily available and widely used in veterinary medicine, leaving the fate of wild *Gyps* vultures in doubt.

**754 Bad for the Gonad: Atrazine is a Gonadotoxin With Consistent Effects Across Vertebrate Classes** T. Hayes, Univ of California – Berkeley, Dept of Integrative Biology. The induction of aromatase and estrogen synthesis in the gonads of alligators was the first finding to suggest that atrazine was an endocrine disruptor in wildlife. This study was followed by field studies that suggested that atrazine is associated with feminized frogs in the wild. Thereafter, multiple laboratories showed that atrazine demasculinizes and feminizes male amphibians, but these findings and/or their significance have been challenged repeatedly by industry-funded scientists over the last ten years. Recently, however, 43 independently funded scientists representing 13 countries reviewed the effects of atrazine across vertebrate classes. Consistent effects in fish, amphibians, reptiles, birds, and mammals were revealed. In particular, the demasculinization and feminization of male gonads via multiple endocrine disrupting mechanisms have been documented and meet all of the so-called "Hill criteria" for establishing cause-effect. As should be expected, the effective doses vary across vertebrate classes with amphibian and fish being the most sensitive to the herbicide (low ppb range), and mammals and especially birds being the least sensitive. In addition, our analysis revealed multiple endocrine disrupting mechanisms including increases in stress hormones, decreases in androgens and increases in estrogens. The effects are produced via interactions at multiple sites along the stress and reproductive axes. New data in amphibians demonstrate that the morphological effects are accompanied by behavioral and functional impairments on reproductive function. Similarly, mammals showed impaired reproductive function as well. The morphological effects have also been associated with amphibians in the wild in multiple studies, suggesting that atrazine may play



a significant role in amphibian declines, but effects on other taxa have not been examined in the wild. To date, regulatory agencies have failed to comprehensively examine the totality of evidence demonstrating atrazine's effects in the laboratory and potential impact on environmental and human health.

**755 Use of Passive Sampling to Measure the Sorption of PCDDs, PCDFs, and PAHs to Black Carbon in Passaic River and Newark Bay Sediments** M.K. Lambert, Univ of Rhode Island. Graduate School of Oceanography, USEPA, OSRTI, Environmental Scientist; R. Lohmann, Univ of Rhode Island, Graduate School of Oceanography, Univ of Rhode Island. The partitioning of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) onto black carbon (BC) strongly controls the fate of PCDD/Fs in the environment and yet few studies have calculated equilibrium partitioning coefficients ( $K_{iBC}$ ) for PCDD/Fs using measurements of in situ contaminated sediments. Laboratory measurements of sorption to BC often differ from field observations due to the effect of different types of BC and of competition with other organic molecules.  $K_{iBC}$  values were derived from measurements of sediments in the Passaic River and Newark Bay, NJ for PCDD/Fs and polycyclic aromatic hydrocarbons (PAHs). An OC partitioning model and a BC-inclusive, Freundlich partitioning model were used to interpret the measured sediment concentrations of OC, BC, PCDD/Fs, and PAHs, and the freely dissolved concentrations of PCDD/Fs and PAHs. A non-depletive, polyethylene passive sampling method was used to measure freely dissolved concentrations at equilibrium, circumventing the need to correct for sorption to dissolved organic carbon. Such corrections introduce uncertainty to the measured porewater concentrations. A time-series, equilibrium experiment indicated the variability for the passive sampling method used in this study was < 15%. The Freundlich model predicted the partitioning of PAHs to within a factor of 2-3 of the observed partitioning, using a Freundlich coefficient  $n=0.7$  and literature  $K_{iBC}$  values. Literature  $K_{iBC}$  values for PCDD/Fs, with a  $n=0.7$ , produced predicted PCDD/F partitioning that differed by as much as 2 orders of magnitude from the observed partitioning. Site-specific  $K_{iBC}$  values were calculated for PCDD/Fs at  $n=0.6$ ,  $0.7$ , and  $0.8$ .  $K_{iBC}$  values calculated at  $n=0.8$  were 10-100 times larger than those calculated at  $n=0.6$ . Based on the close fit of the PAH partitioning using  $n=0.7$ , it was judged that this is most appropriate Freundlich coefficient for PCDD/Fs as well. The calculated  $K_{iBC}$  values range up to 2 orders of magnitude larger than those derived from a study of HOC partitioning in Boston Harbor, but are 2 to 4 orders of magnitude smaller than those observed in the more pristine environment of Baltic Sea sediments. Unexpectedly, PCDDs displayed stronger sorption than PCDFs or PAHs relative to their aqueous solubilities. Although the presence of BC in the sediments reduced the overall bioavailability of PCDD/Fs by > 90%, the sediments at 2 m depth in the Passaic River display high porewater activities of PCDD/Fs.

**756 Measurement and Estimation of PCB Porewater Concentrations and Release of PCBs from Sediment to Water in Indiana Harbor and Ship Canal** A. Martinez, Univ of Iowa, Dept of Civil & Environmental Engineering, and IHR-Hydrosience and Engineering; D. Reible, Univ of Texas, Environmental and Water Resources; K.C. Hornbuckle, Univ of Iowa, Dept of Civil & Environmental Engineering. Sediments contaminated with persistent, bioaccumulating and toxic compounds (PBTs) are an important source of these chemicals to the environment. PBT release from sediments is caused by both episodic resuspension and continuous soluble release from the bed sediment. The latter is a predictable function of the sediment porewater concentration, although this is a measurement that is difficult for PBTs because of their relatively low water solubilities. Measurements of PCBs freely dissolved porewater concentrations from Indiana Harbor sediment ranged from 100 to 500 ng L<sup>-1</sup> using a novel passive sampler technique (SPME PDMS-fiber). It was possible to determine ~ 80 individual or coeluting congeners in the porewater. Isotherm experiments showed that less than 10 days were enough to achieve equilibrium between the PDMS-fiber and the sediment porewater concentration. Total organic carbon, organic carbon and black carbon were measured. High levels of black carbon as a fraction of total organic carbon were found (median ~30%), which reflect the long history of local combustion sources. Dissolved organic carbon (DOC) was also measured in the core sample and ranged from 30 to 300 mg L<sup>-1</sup>, and it is strong correlated with core depth. A partitioning approach between PCBs and organic and black carbons yielded the best approach to calculate PCB porewater concentration in the sediment from bulk PCB concentrations. A release PCB concentration was calculated

from the PCB porewater (measured and calculated) and the DOC values, and was used to estimate the release of PCBs from the sediment into the overlying water. Black carbon was also incorporated in the calculation of the mass transfer coefficient.

**757 Procedures for Derivation of Site-specific Equilibrium Partitioning Sediment Benchmarks for the Protection of Benthic Organisms: Nonionic Organics** S. Kane Driscoll, Exponent Inc.; S. Ahn, Exponent Inc., Postdoctoral scholar; J. Pietari, Exponent Inc.; R. Burgess, USEPA, ORD/NHEERL Atlantic Ecology Division. This presentation provides an overview of draft guidance being developed by USEPA (EPA) on procedures that can be used to modify standard equilibrium partitioning sediment benchmarks (ESBs) for nonionic organic chemicals to reflect site-specific conditions. The procedures used to develop site-specific ESBs are intended to supplement the procedures described for standard ESBs based on the equilibrium partitioning (EqP) theory. EqP theory holds that nonionic chemical, such as PAHs, partition between sediment organic carbon, interstitial (pore) water, and benthic organisms. EPA currently recognizes that the standard ESBs may be under- or overprotective if the bioavailability of a chemical differs in site sediments because of additional partitioning phases (e.g., soot or black carbon). The site-specific procedures described in the guidance document assume that the concentration of bioavailable chemical can be reasonably measured or estimated from the concentration of freely dissolved chemical in interstitial water. The guidance includes examples that demonstrate the calculation of site-specific ESBs using various approaches including: a "two-carbon" model that estimates the concentrations of chemicals in interstitial water by taking into account the influence of black carbon, direct measurement of chemicals in isolated samples of interstitial water, and concentrations of chemicals in interstitial water measured using passive samplers. Consistent with the recommendations of EPA's Science Advisory Board, the guidance does not imply that ESBs should be used as stand-alone, pass-fail criteria for all applications; rather, ESB exceedances can be used to trigger the collection of additional assessment data. A potential tiered approach for implementing site-specific ESBs while balancing environmental protection and cost is presented. The first tier uses the standard EqP-based ESBs to assess the likelihood of toxicity to the benthos. In the second tier, passive samplers can be used to generate site-specific ESBs. If the site-specific ESB is exceeded, there is the option to conduct sediment toxicity testing to verify the findings of the first two tiers. Site-specific ESBs may be useful as a complement to existing sediment assessment tools, to help evaluate the extent of sediment contamination, to identify chemicals causing toxicity, and to serve as targets for pollutant loading control measures.

**758 Contrasting PCDD/F and PBDE Activities in Biota, Sediment, and the Water Column of Newark Bay, New Jersey (USA)** C. Friedman, MIT, Center for Global Change Science; R. Lohmann, Univ of Rhode Island, Graduate School of Oceanography, Univ of Rhode Island. The bioavailability of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polybrominated diphenyl ethers (PBDEs) was determined at five sampling sites in Newark Bay, New Jersey (USA). Deposit- and filter-feeding organisms were collected from the sites and their tissues were analyzed for PCDD/F and PBDEs. Freely dissolved PCDD/F and PBDE porewater concentrations were determined in the laboratory using polyethylene (PE) passive samplers. Freely dissolved porewater PCDD/F and PBDE concentrations were also back-calculated based on their partitioning to sedimentary organic carbon (OC), and black carbon (BC) assuming equilibrium partitioning. Lastly, PE samplers were also deployed in situ to measure freely dissolved deep water PCDD/F and PBDE concentrations. All freely dissolved concentrations were compared to those based on lipid uptake. For PCDD/Fs, PE-derived porewater and/or deep water concentrations were generally closest to those estimated from lipid. For PBDEs, porewater, deep water, and sediment OC alternated in most closely predicting tissue exposures to PBDEs. Considering BC sorption of PBDEs caused underestimation of organism exposures by at least an order of magnitude. Lower molecular weight (MW) PCDD/Fs were approximately at equilibrium between porewater and deep water, whereas higher MW congeners demonstrated a gradient from the porewater to the water column. Low MW PBDEs generally had activity gradients in the direction of the water column. The direction of high MW PBDE gradients depended on the congener and site. Results indicated that PE has potential to measure bioavailability accurately for deposit-feeding organisms in the field, and that BC sorption

may play a less important role in determining PBDE bioavailability than for PCDD/Fs.

**759 Biodynamic Modeling of Remedial Success of In Situ Activated Carbon Amendment to Sediment and Possible Secondary Effects on Benthic Invertebrates** E.M. Janssen, Stanford Univ, graduate student, Stanford Univ; R.G. Luthy, Stanford Univ, Civil and Environmental Engineering. The primary goal of sediment remediation is to reduce risk to ecosystems and humans by reducing contaminant release to overlying water and exposure to biota. A secondary goal is that the remedial approach should allow ecosystem recovery, e.g., the restoration of the benthic community. Our previous work showed that at full-scale, in situ sorbent amendment with activated carbon (AC) may reduce bioaccumulation of polychlorinated biphenyls from sediment by up to 85 to 90% under favorable field and treatment conditions. We demonstrate a biodynamic modeling framework for benthic organisms with different feeding strategies to assess how the remedial success of a sorbent amendment that lowers the contaminant availability can be compared to reference conditions and traditional cleanup goals, which are commonly based on bulk sediment concentrations. In situ AC amendment is a promising yet novel sediment remediation alternative and possible secondary effects on benthic invertebrates by sorbent addition need to be better understood. The presence of ingestible and non-ingestible AC in three reference sediments slightly increased weight loss but had no effect on survival, lipid, glycogen or protein content of *Neanthes arenaceodentata*, a deposit feeding polychaete, which does not discriminate against AC in its diet.

**760 Reducing PAH (Bio)availability by In Situ AC Amendment to Soils and Sediments** S. Hale, Norwegian Geotechnical Institute; G. Cornelissen, Norwegian Geotechnical Inst; E. Eek, G. Breedveld, K. Amstatter, M. Elmquist, Norwegian Geotechnical Institute; T. Hartnik, L. Jakob, Soil and Environment Division, Norwegian Institute for Agricultural and Environmental Research (Bioforsk); T. Henriksen, Lindum Ressurs og Gjenvinning AS; J. Gunnarsson, J. Hedman, G. Samuelsson, Stockholm Univ, Dept of Systems Ecology; O. Stokland, Marine Bunnndyr AS. Activated carbon (AC) has a very high sorption capacity for organic pollutants and when added to contaminated soil or sediment can sequester and reduce the (bio) availability of these pollutants. We present a series of field AC amendments in Norway in which PAH-contaminated soils and sediments have been treated. A variety of innovative analysis techniques were used to assess the effectiveness of the amendment, such as the deployment of in situ porewater passive samplers and benthic diffusion flux chambers. At Trondheim harbor, AC was applied as a thin layer cap to a marine underwater sediment. Caps consisting of AC-alone, AC+clay and AC with a sand layer on top were compared to sand only and no cap. Underwater imaging showed successful cap placement and up to 60 % of the AC was recovered for the AC+clay cap. The sediment-to-water PAH flux was reduced for all AC treatments and the greatest reduction (factor of 10) was observed for the AC+clay. Porewater PAH concentrations were reduced up to 50 % in the biologically active 0-5 cm deep sediment layer which contained AC. Bioaccumulation of PAHs to worms and benthic mussels decreased 80-90% for the AC+clay cap. AC+clay was recommended as the best amendment method because it showed; i) best AC recoveries; ii) best chemical and biological effectiveness; iii) lowest detrimental secondary effects to the benthic communities. At Drammen, powdered and granular (PAC and GAC) AC were added to a soil. Freely dissolved aqueous PAH concentrations in drainage water and soil pore water were monitored with passive samplers. More than one year after amendment, the free aqueous concentrations in the drainage water were reduced by 93 % and 55 % for the PAC and GAC, respectively, and the free aqueous PAH concentration in the soil pore water itself, as measured by innovative dug-in passive samplers, was reduced by 93 and 70 % for PAC and 84 and 61 % for the GAC. The secondary chemical effects of the AC amendment were considered by monitoring the concentration of DOC and nutrients. Both PAC and GAC bound DOC while the concentrations of nutrients were variable and likely affected more by external environmental factors than the AC amendment. Biological testing showed that the amendment of 2% PAC had a negative effect on plant growth, but GAC actually improved growth. PAC was toxic to earthworms as it reduced the worm's weight. Both kinds of AC significantly reduced Biota to Soil Accumulation Factors (BSAFs) in earthworms and plants.

**761 Activated Biochars for the In Situ Sequestration of Organics, Mercury and Carbon in Sediments** J.L. Gomez-Eyles, Univ of Maryland Baltimore County; B. Beckingham, Univ of Maryland Baltimore County, Civil & Environmental Engineering, Univ of Maryland Baltimore County, Dept of Civil & Environmental Engineering; S. Kwon, UMBC; U. Ghosh, Univ of Maryland Baltimore County, Civil & Environmental Engineering. In aquatic environments that are impacted by contaminated sediments, contaminant transport pathways can be interrupted by enhancing the binding capacity of natural sediments. This study evaluates the potential of a series of especially formulated biochars (derived from pine wood, peanut hull, barley straw and acai pit) and activated poultry litter biochars to sequester organic and metal contaminants in sediments, while reducing or even reversing the carbon footprint of sediment remediation efforts. PAH, PCB and DDT isotherm studies were conducted at environmentally relevant concentrations using polyoxymethylene solid-phase extraction (POM-SPE), to evaluate the sorption capacity of the different biochars. Freundlich isotherms were constructed and biochar performance was compared with that of commercially available activated carbons (CACs). Biochars were effective at sorbing organics with Kf values ranging from 6.4 to 7.2 for PCB 47, a mid-range tetra-PCB. This sorption was highly non-linear, with n values ranging from 0.53 to 0.81 for PCB 47. However, CACs consistently removed more PCBs from solution, followed by the activated poultry litter biochars suggesting surface area is a key parameter controlling organic contaminant sorption potential. The difference in sorption potential between CACs and biochars was greater for the less chlorinated PCBs with lower Kow. Sorption studies were also carried out for mercury at a range of pH concentrations (pH 3-11). The activated poultry litter biochars removed >99% mercury from solution over the whole pH range, whilst the CACs removed between 18 and 95% depending on pH level. This suggests the surface functionality of the biochars make them more effective for mercury removal, than the CACs. A better understanding of how biochar characteristics affect contaminant sorption is necessary to improve biochar quality and to help in the selection of the appropriate biochar amendment necessary to achieve site specific contaminated sediment remediation goals.

**762 Biochar-Herbicides Antagonism: Can They Stand Each Other?** A. Freddo, Univ of East Anglia; B. Reid, Univ of East Anglia, Environmental Sciences. The benefits on plant growth and improvement of soil structure following biochar addition to soil have been previously reported. However the physic-chemical structure of biochar allows the sorption of organic compounds (for example pesticides), thus, while on one hand biochar might increase crop yields the beneficial role of pesticides might be undermined through alteration of their bioavailability to pests and weeds. The purpose of this study was to establish the efficacy of four herbicides (mesotrione, pendimethaline, terbuthilazine, isoproturon) upon three common broadleaf weeds (*Amaranthus retroflexus*, *Solanum nigrum* and *Abutilon theophrasti*) when applied to soil amended with 0%, 1% and 5% biochar. The results indicated that in all the treatments the presence of higher concentration of biochar in soil significantly reduced the effectiveness of all herbicides tested. The viability of targeted weeds in the herbicide amended 5% biochar soil was not significantly ( $p < 0.05$ ) different to the number of viable weeds observed in the control treatments (no biochar and no herbicide). Physiologic characteristics were also taken into account. Results regarding stem- and root-length and fresh weight of biomass indicated that weeds present in the 5% biochar plus pendimethaline treatments were not significantly ( $p < 0.05$ ) different to the control. Thus, biochar was found to be effective in reducing the bioavailability of the dissimilar herbicides to contrasting broadleaf weeds. These results are significant in so much as they suggest biochar incorporation to soil could undermine food security on account of the potential for biochar to deactivate herbicidal activity.

**763 An NMR-based Metabolomic Analysis of Cobia Health in Response to Dietary Manipulation** T. Schock, National Institute of Standards and Technology, Analytical Chemistry Division; S. Newton, Univ of Arkansas, Pine Bluff; K. Brenkart, J. Leffler, South Carolina Dept of Natural Resources; D. Bearden, National Institute of Standards and Technology, Analytical Chemistry Division, National Institute of Standards and Technology. Commercial aquaculture feeds rely heavily on fishmeal and fish oil, which can be expensive and ecologically unsustainable. The environmental impact of various aquaculture practices because of the need to harvest fish for fish meal and the need to properly handle the waste stream in intensive aquaculture systems leads to investigation of alternative, plant-based foods.

To evaluate the efficacy of reduced fishmeal diets for outgrowth, a dietary study was conducted on the finfish cobia, *Rachycentron canadum*. Juvenile cobia were raised on four different diets: a commercial diet (Control), a diet with conventional levels of fishmeal (FM100), a diet with a 50% reduction in fishmeal (FM50), and a diet with 25% of the conventional level of fishmeal (FM25). The experimental diets substituted varying amounts of soybean meal and poultry meal for fishmeal. NMR-based metabolomic techniques were used to assess the effect of decreasing dietary fishmeal on the health of the cobia. Filtered serum  $^1\text{H}$  NMR spectra analyzed by principal components analysis (PCA) showed cobia fed reduced fishmeal diets were metabolically different than cobia on control diets. In particular, tyrosine and betaine increased in cobia fed reduced fishmeal diets while glucose decreased, suggesting that these cobia were not receiving the necessary nutritional components required for energy and growth. The formulated control diet contributed to enriched growth and significantly elevated lactate levels suggesting enhanced gut microflora metabolism in response to dietary components. For intensive fish production a natural antibiotic/probiotic enhanced by diet would be ideal, limiting the need for prophylactic antibiotics and addressing the concern of additives in food for human consumption. In addition, these metabolomic studies have laid a foundation for studying the population of wild fish that may be impacted by naturally occurring diet variation in the natural fisheries. The results show that NMR-based metabolomic analysis is a useful tool in aquaculture studies and organism health assessment.

**764 Comparison of the Metabolic Responses Elicited via Exposure to Crude and Dispersed Oil Dissolved Components** A. Van Scoy, Univ of California, Davis, Dept of Environmental Toxicology, Univ of California-Davis, Environmental Toxicology, Univ of California, Dept of Environmental Toxicology; B. Anderson, Univ of California – Davis, Dept of Environmental Toxicology; B. Phillips, Univ of California – Davis, Environmental Toxicology; J. Voorhees, University of California – Davis, MPSTL – Environmental Toxicology; R. Tjeerdema, Univ of California – Davis, Environmental Toxicology. Crude oil contamination remains a problem along coastal California due to its demand and major economic importance. Resource managers require information on the acute toxicity of treated and untreated oil, and their sublethal effects in wildlife. Investigation of the damage hydrocarbons pose towards marine organisms is becoming more involved, particularly thru the use of metabolomics, which has the potential to examine metabolic responses of organisms exposed to environmental and anthropogenic stressors. This study examined the toxic effects of the water-accommodated fraction (WAF) and the chemically-enhanced WAF (CEWAF, with Corexit 9500) of Prudhoe Bay Crude Oil (PBCO) on adult topsmelt (*Atherinops affinis*) and pre-smolt Chinook salmon (*Oncorhynchus tshawytscha*) using NMR-based metabolomics. Muscle samples were analyzed following 96-h exposures to either WAF or CEWAF and principal component analysis (PCA) was applied to identify variation between samples and treatments as a result of exposure. Based on the LC50 values, toxicity to both topsmelt and salmon decreased (i.e., less toxic) when Corexit 9500 dispersant was applied. However, NMR profiles and metabolite responses for both fish species were similar for both WAF and CEWAF, thus suggesting the fish were accumulating similar dissolved hydrocarbon concentrations. Therefore, semi-permeable membrane devices (SPMDs), which specifically accumulate dissolved components within water, were used to confirm this hypothesis. It was found that SPMDs did accumulate two- and three-ringed PAHs at similar concentrations, following exposures to either WAF or CEWAF. Thus it appears that similar hydrocarbon concentrations are eliciting the similar metabolic responses. Since SPMDs specifically target dissolved components, the variation among the measured LC50 values is possibly due to the combined analysis of dissolved and particulate oil (as they are difficult to separate). Overall, NMR-based metabolomics was successful in assessing the sublethal effects of petroleum hydrocarbons on topsmelt and salmon. More importantly, the metabolomics approach has led to confirmation that dissolved (bioavailable) hydrocarbon concentrations, those likely responsible for the toxic actions, are similar in both WAF and CEWAF.

**765 Cell-based Metabolomics: A New Tool for Environmental Toxicology** Q. Teng, USEPA, National Exposure Research Laboratory, National Exposure Research Lab, USEPA, National Exposure Research Laboratory; D. Ekman, T. Collette, USEPA, National Exposure Research Laboratory. Metabolomics is a powerful 'omic tool that has great potential for ranking

and screening large lists of chemicals for exposure and effects, and for developing biomarkers for both human and ecological exposures. The traditional chemical screening methods using rodents as test species are not sustainable in the long term, given the ever increasing number of chemicals that must be tested for safety. The most promising approach to increase the throughput of metabolomics is to use cell cultures instead of live animals. This is consistent with the bold vision laid out by the National Research Council in "Toxicity Testing in the 21<sup>st</sup> Century". We have made major developments that position cell-based metabolomics as an important, viable, and sustainable approach for rapid chemical screening and prioritization and for developing biomarkers.

**766 Earthworm Sub-lethal Responses to Polychlorinated Biphenyls in Freshly Spiked vs. Historically Contaminated (>30 years) Soil Using  $^1\text{H}$  NMR Metabolomics** M. Whitfield Aslund, Univ of Toronto, Physical and Environmental Sciences; M.J. Simpson, A.J. Simpson, Univ of Toronto Scarborough, Dept of Physical and Environmental Sciences.  $^1\text{H}$  NMR metabolomics was used to measure earthworm sub-lethal responses to polychlorinated biphenyls (PCBs) in freshly spiked and historically contaminated soils. A preliminary study examined the metabolic profile of *Eisenia fetida* earthworms exposed to an artificial soil spiked with sub-lethal concentrations of PCBs (0.5 – 25 mg/kg Aroclor 1254). After only two days of exposure,  $^1\text{H}$  NMR metabolomics provided evidence that PCBs disrupted both energy systems (ATP levels) and membrane functions (betaine levels) in *E. fetida*, which matched well with known toxic mechanisms of action for PCBs. As traditional methods for assessing sub-lethal toxicity of soil contaminants to earthworms require much longer periods of soil exposure (e.g., the earthworm reproduction test requires >50 days), this suggested that metabolomics might provide a more rapid alternative. However, as this soil was freshly spiked, PCB bioavailability likely exceeded that of an aged soil. Soil aging is believed to reduce contaminant bioavailability and toxicity with time and it was unclear how this would alter the earthworm metabolomic response. Therefore,  $^1\text{H}$  NMR metabolomics was also applied to assess earthworm responses to aged (>30 years) PCB-contaminated field soils. Earthworms exhibited a less defined metabolic response to PCBs in this aged soil than was previously observed for freshly spiked soil, even though the total PCB soil concentrations were higher (91-280 mg/kg Aroclor 1254/1260). For example, PLS regression suggested a stronger relationship between the earthworm metabolic profiles and soil PCB concentration for the spiked soil after two days exposure (cross-validated PLS-regression with 7 components,  $R^2X=0.99$ ,  $R^2Y=0.77$ ,  $Q^2Y=0.45$ ,  $p<0.001$ ) than for the aged soil after two days exposure (cross-validated PLS-regression with 2 components,  $R^2X=83.4\%$ ,  $R^2Y=22.6\%$ ,  $Q^2Y=10.3\%$ ,  $p=0.004$ ). The comparison between freshly spiked and aged PCB-contaminated field soils indicates that  $^1\text{H}$  NMR metabolomics of earthworm responses is sensitive to both contaminant concentration and bioavailability. These results also suggest that earthworm metabolomics may be a sensitive tool for risk assessment of contaminated sites.

**767 Effects of Oil Dispersions on Key Species in the Atlantic Ocean, Cod *Gadus morhua* L. and Copepod *Calanus finmarchicus*, Studied by NMR Metabolomics** T.R. Storseth, SINTEF Fisheries and Aquaculture AS, Marine Resources Technology; B. Hansen, SINTEF Materials & Chemistry, Marine Environmental Technology; K.F. Degnes, SINTEF Materials and Chemistry, Biotechnology; A.J. Olsen, Norwegian Univ of Science and Technology, Biology; D. Altin, BioTrix; T. Nordug, SINTEF Materials and Chemistry, Marine Environmental Technology. Oil related activity in the North Atlantic Ocean demands risk assessment that includes effect studies on relevant species. Spawning grounds for Atlantic cod, *Gadus morhua* L., are being considered for oil exploration and knowledge on the effects of oil spills on cod larvae is important. In addition, with an annual production of 300 million tons, and as such a considerable source of food for the North Atlantic fish species, the marine copepod *Calanus finmarchicus* is a highly relevant species for risk assessment. We have used  $^1\text{H}$ -NMR metabolomics to study the effects on the metabolite composition of exposed cod larvae and *C. finmarchicus* to mechanically (MD) and chemically dispersed (CD) oils. concentrations (given in sum of PAH including Naphthalenes in  $\mu\text{g/l}$  for MD and CD, respectively) were, low (0.08, 0.66), medium (1.36, 2.32) and high (8.43, 8.71). In addition, since we have previously documented oil-induced loss of appetite in cod larvae, we also included a group of cod larvae which was starved in order to compare with the groups exposed to dispersed oil. Cod larvae were exposed to dispersed oil for 96 hrs at which time they were



sampled and analyzed. The metabolic changes were described by principal component analysis (PCA). The high concentration chemical dispersion group was found to be different from the control (Wilcoxon rank sum test on scores from PC1  $p < 0.05$ ) whereas the medium and low dose groups from chemical and mechanical dispersion did not differ from the control group. Starvation for 96 hours induced larger but similar changes compared to the CD high dose group. *C. finmarchicus* was sampled before and after a 96 hr exposure and in addition after a 96 hr recovery period. Metabolite response trajectories for exposure to MD and CD oils in were based on 2D scores from PCA. Changes in the two PCs show three traits. Low concentrations seem to cause reversible effects compared to control groups, whereas medium and high doses show changes along PC1 after exposure which are reversed after recovery. Along PC2 the medium and high doses showed changes that were not reversed after the recovery period. Thus the 2D scores plot and corresponding loadings may describe reversible and irreversible effects of exposure. A discussion will be given on observed changes in amino acids, glucose and osmolyte levels for both species. Cod data will be compared to previously presented data on effects of exposure to chemically dispersed oil vs. water soluble fractions.

**768 Exposure to Elevated Levels of CO<sub>2</sub> Induces a Shift in Metabolic Fingerprint in Different Tissues and Hemolymph in the Shore Crab *Carcinus maenas*** K.M. Hammer, Norwegian Univ of Science and Technology, Biology; T.R. Storseth, SINTEF Fisheries and Aquaculture AS, Marine Resources Technology; S.A. Pedersen, Norwegian Univ of Science and Technology, Biology. Carbon dioxide acts as a weak acid when dissolved in water, and the increasing level of CO<sub>2</sub> in the atmosphere may therefore lead to an acidification of the ocean. Further, mitigating alternatives such as storing CO<sub>2</sub> in rock formations under the seafloor may result in leakage of the gas into the water column. In the recent years more focus has therefore been turned towards studying the effects of elevated levels of seawater CO<sub>2</sub> (hypercapnia) on marine fish and invertebrates. Until now the studies have mainly focused on the effect on acid-base balance, metabolic rate, development, growth and calcification. We have looked into the metabolic response of the shore crab *Carcinus maenas* to time and dose dependent exposure to environmental hypercapnia by analyzing water soluble metabolites of gill, leg muscle and hemolymph using <sup>1</sup>H nuclear magnetic resonance spectroscopy. Animals were subjected to short-term (1-96 hours) and prolonged (1-4 weeks) exposure to hypercapnic seawater (~1% pCO<sub>2</sub>) at pH<sub>NBS</sub> 6.95, as well as exposure to pH<sub>NBS</sub> 7.40, 6.95, 6.60 and 6.35 (-0.5, 1, 2 and 3% pCO<sub>2</sub>) for two weeks. Multivariate analysis of NMR data showed an increasing difference in the metabolic profiles of tissues and hemolymph with increasing CO<sub>2</sub> levels compared to control, while difference was only found after prolonged exposure in the time series study. The changes in metabolic response were mainly attributed to betaine, glycine, lactate, glutamate, glutamine, proline, alanine and sarcosine. The results indicated that both tissues and hemolymph were suited for <sup>1</sup>H-NMR analysis of metabolic response in *C. maenas* subjected to hypercapnic conditions, and that the changes mainly occurred after prolonged exposure.

**769 Metabolomics for Biomonitoring of Contaminants of Emerging Concern in the Great Lakes** T. Collette, USEPA, National Exposure Research Laboratory; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; E. Durhan, USEPA, Mid-Continent Ecology Division; D. Ekman, USEPA, National Exposure Research Laboratory; K.M. Jensen, M. Kahl, USEPA, Mid-Continent Ecology Division; K. Lee, USGS; E. Makynen, L. Thomas, USEPA, Mid-Continent Ecology Division; A. Trowbridge, US Fish and Wildlife Service; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division. Metabolomics (and other 'omic techniques) are now well established for studying contaminant-induced alterations to normal biological function. These alterations, typically observed in laboratory-controlled studies, have been used successfully to screen for chemical potency, or to infer a chemical's toxic mode-of-action. But, the use of these techniques for biomonitoring in the natural environment, while often stated as an ultimate goal, has rarely been demonstrated to add value in chemical exposure assessment. We have recently conducted biomonitoring studies in the St. Louis Harbor, Lake Superior, in support of the Great Lakes Restoration Initiative (GLRI), using caged fathead minnows (*Pimephales promelas*). Fish were deployed at four field sites including in the upper St. Louis River, and at locations near wastewater treatment facilities (both proximal and distal to site of discharge). A

variety of endpoints were measured in the fish, including targeted gene expression and NMR-based metabolite profiles. In addition, water from these sites was analyzed for an extensive list of contaminants of emerging concern. Metabolomics appears to be particularly useful in this study for characterizing the fishes' exposure to chemical contaminants. For example, the extent of perturbation of hepatic metabolite profiles in females (compared to laboratory controls) generally follows patterns expected based on extent of contamination. Additionally, the availability of extensive chemical monitoring data will help lead to an understanding of the connection between the presence of specific contaminants and specific metabolite perturbations, yielding biomarkers of exposure that can be used in other field applications.

**770 Studying the Physiology of Declining *Diporeia* Populations in the Laurentian Great Lakes Using Metabolomics** S. Maitry, Purdue Univ, graduate student; A. Jannash, Purdue Univ, Bindley Bioscience Center at Discovery Park; J. Adamec, Univ of Nebraska, Dept of Biochemistry, Beadle Center N151; T. Nalepa, National Oceanic and Atmospheric Administration (NOAA), Great Lakes Environmental Research Laboratory; T.O. HOOK, M.S. SEPULVEDA, Purdue Univ, Dept of Forestry and Natural Resources. Since the 1990's, *Diporeia* populations have declined across the Great Lakes region except Lake Superior. Several hypotheses have been put forward to help explain this precipitous decline including introduction and establishment of dreissenid mussels, limited food availability, pathogens, and exposure to contaminants. We applied mass spectrometry-based metabolomics (GC X GC/ TOF-MS and LC-MS) to study the metabolome of *Diporeia* exposed to different stressors through a series of laboratory experiments. *Diporeia* responded with stressor-specific changes in metabolic pathways: 1) a 60 d starvation resulted in altered proline-alanine-glutamate and glycerophospholipid metabolism; 2) co-exposure to quagga mussels affected serine, threonine and glycine metabolism; and 3) exposure to PCB-contaminated sediments induced changes in glutamine and phenylalanine metabolism as well as hydrocarbon receptor mediated pathways. We are currently analyzing the metabolome of ~ 100 *Diporeia* collected from stable and declining populations across the Great Lakes region. Preliminary results indicate a significant "Lake Effect" on the metabolite profile of *Diporeia*. We hope the combined results of our laboratory and field metabolomic studies will help elucidate the potential cause(s) of *Diporeia* declines in the Great Lakes.

**771 Temporal Aspects of Water Use in Shale Gas Development: Implications for LCA** S.M. Jordaan, Harvard Univ, Dept of Earth and Planetary Sciences, Energy Technology Innovation Policy group; D.P. Schrag, Harvard Univ, Dept of Earth and Planetary Sciences, Dept Environmental Science and Engineering. Life cycle assessment (LCA) has been criticized for temporal aggregation of resource use over the life of a product in the development of inventories. This may have implications for energy transitions when resource requirements change over the life of energy extraction projects. Shale gas is expected to become a large contributor to US energy production over the coming years, with forecasts indicating shale gas may contribute 45% of natural gas production by 2035. Water use implications of increased levels of shale gas production are not well studied, yet they are clearly different from conventional natural gas due to the use of hydraulic fracturing. Previous work has estimated the water consumption for shale gas extraction to be small (0.6 – 1.8 gal/MMBtu) when compared to coal extraction (5 – 15 gal/MMBtu). Hydraulic fracturing, however, typically requires water use prior to production, resulting in a heavy front-loading of water consumption. As water may be scarce in some regions, this front-loading may have implications for water availability and limitations to the rate of expansion of drilling activity. Preliminary estimates indicate that using temporally aggregate water consumption in forecasting water use for shale gas development may result in underestimating the water use by as much as a factor of 3. This indicates a need to develop systematic methods to consider temporal aspects within LCA. A method is proposed to account for the temporal front-loading of water use in shale gas development within LCA, where marginal water use is calculated, rather than the average water use over the life of a product. Life cycle water consumption for electricity generated with shale gas under both methods will be compared to other electricity generation options. This study suggests the use of temporally aggregate assumptions can decrease the accuracy of impact assessment models, particularly for resources with limited availability.

**772 Potential Land and Water Impacts Related to Increased Biofuel** J.C. Bare, USEPA, MS-466 There is no international consensus on how to conduct land and water use impact assessment for LCIA, yet decisions are currently being made which have the potential for impacts in these areas. The increased agricultural production for the manufacture of biofuels in the US is an issue which could have widespread impacts on water use, irrigation, and aquifer depletion as well as many other land use issues. This paper will provide a LCIA methodology for evaluating water use/depletion impacts with US corn-to-ethanol as a case study. Land use can also cause numerous impacts on the future usability of the land for agriculture and other purposes. Using an ecosystem services tool and GIS inputs available from the USEPA's Future Midwest Landscape Project, a framework will be provided for displaying land use analysis comparing a 2002 land use scenario to a projected 2020 land use scenario, where the principle driver for change is the increased corn production necessary to produce additional ethanol. The potential for increased (or decreased) erosion will be displayed for a 12 state region which makes up the primary corn-to-ethanol production. An additional map will be generated which shows the potential for erosion in the current land use pattern.

**773 Dynamic Multi-crop Model to Characterize Impacts of Pesticides in Food for LCIA** P. Fantke, Univ of Stuttgart, IER; R. Juraske, Swiss Federal Institute of Technology Zurich; A. Anton, IRTA Carretera de Cabrils; R. Charles, Agroscope Changins-Wädenswil; O. Jolliet, Univ of Michigan, School of Public Health. Human intake of pesticide residues via ingestion of processed food plays an important role for evaluating current agricultural practice. A new dynamic plant uptake model – dynamiCROP – is presented to determine intakes of pesticides residues. The model is based on a set of interconnected compartments customized to six crops (wheat, paddy rice, tomato, apple, lettuce, potato). Modeled residues are compared with measured concentrations of eleven different pesticides. Measurements and model estimates correspond well with total crop-specific residual errors the difference ranging between a factor 1.5 for lettuce up to a factor 37 for rice with an overall residual error a factor 32 over all 12 substance-crop combinations. Intake fractions and related impacts were calculated per unit mass of applied pesticide for 121 substances applied to the six crops. For all crops but potato, intake fractions are usually in the range of  $1\text{E-}02$  and  $1\text{E-}08$  kgintake/kgapplied for typical times between application and harvest. The highest intake fraction are observed for insecticides and fungicides applied to tomatoes and lettuces. The intake fractions obtained after direct application were one to five orders of magnitude higher than intake fractions estimated by USEtox for indirect emissions. The main factors influencing the fate behavior of pesticides are the degradation half life in plants, in plant surface, the residence time in soil surface as well as the time between pesticide application and harvest. A simplified model based on these parameters enables prediction of residues within a factor 10 of those calculated with the detailed model, with  $R^2$  higher than 0.8; this simplified model is adequate to assess direct residues for multimedia models such as USEtox. We demonstrate that toxicity potentials can be reduced up to 99% by defining adequate scenarios for pesticide substitution.

**774 A Methodology for Inclusion of Terrestrial Ecotoxic Impacts of Metals in Life Cycle Impact Assessment** M. Owsianiak, R.K. Rosenbaum, M.Z. Hauschild, Technical Univ of Denmark, Dept of Management Engineering. Terrestrial ecotoxicity is in most cases not addressed or to a very limited extent in life cycle assessment (LCA). We are developing a new method for calculating terrestrial ecotoxicity characterization factor (CF) of metals for application in life cycle impact assessment (LCIA). The method takes into account metal speciation and interactions with soil organic constituents, because these mechanisms control metal bioavailability and influence their toxic properties. Transfer functions and geochemical speciation models are employed to calculate reactive and available fractions of metals in 1300 soils spanning a wide range of properties and pore water chemistry. Site-specific fate factors (FF), bioavailability factors (BF) and effect factors (EF) are then calculated for these soils. The biggest variability is observed for BF, which can vary from 2 to 6 orders of magnitude for the cases of Ni and Cu, respectively. These variations are a result of variability in soil properties such as pH, organic carbon or clay content. Published terrestrial biotic ligand models (TBLM) and free ion activity models (FIAM) are next employed in order to derive terrestrial ecotoxicity EFs. Median EFs predicted with TBLMs for Cu and Ni correspond to average ecotoxicity (range) of 12.4 (6.6 – 364) and 1194 (62 – 42164)  $\mu\text{g/L}$ , respectively. EFs

derived with FIAMs turn out to be 6.5 (Cu) and 7.5 (Ni) times higher than these derived with TBLMs. The ecotoxicity ratio of Cu to Ni is accurately predicted with both models and the contribution of EF to the CF is within the same order of magnitude or lower comparing to that of the BF. Thus, FIAMs can be employed to calculate EFs for metals for which TBLMs are not available. From a set of spatially explicit CFs, site-generic CFs can be derived at global or continental scales. For applications in LCIA, the tradeoff between the level of geographical detail and the level of uncertainty in both spatially explicit and site-generic CFs remains to be investigated. The method highlights the importance of taking into account variability of soil properties in deriving operational characterization factors for terrestrial ecotoxicity of metals.

**775 Including Metal Speciation in LCA: The Case of Terrestrial Ecotoxicity** G. Plouffe, Ecole Polytechnique de Montreal, CIRAIG; C. Bulle, CIRAIG, Ecole Polytechnique de Montreal, Chemical engineering Dept; L. Deschenes, CIRAIG, Chemical Engineering. At present, ecotoxicological impacts of metals are often the main contributors to LCA results. This domination does not necessarily reflect a real toxicity but rather indicates the misuse of fate and effect models, which were initially developed for organic compounds. Characterization factors are often obtained using the total emitted concentration as being the toxic concentration, which is inappropriate for metal emissions because of metal speciation. A framework for including metal speciation in freshwater ecotoxicological impact assessment has been developed recently. It relies on the use of a commercially available geochemical model to define the bioavailable metal fraction. Although really interesting, this approach is not directly applicable to soils, mainly because geochemical speciation models were developed for aquatic environments. Soils heterogeneity hinders generalization and could invalidate the hypothesis made in aquatic geochemical modeling. The main goal of this project is thus to develop a method and speciation model specific to soils in order to obtain terrestrial ecotoxicity characterization factors. According to the framework proposed for freshwater ecotoxicity, a bioavailable factor is added to the definition of the characterization factor. This allows determining fate and effect of metals only on the bioavailable fraction, which is the real responsible for toxicity. The bioavailable fraction in soils is computed with a new speciation model specific to soils developed in this project. The speciation model for soil groups the theoretical basis of two available geochemical aquatic speciation models (WHAM 6.0 and MINEQL+) in order to consider organic complexation reactions as well as reactions with inorganic ligands. New characterization factors for Zn, Ni and Cu are obtained for terrestrial ecotoxicity. Their spatial variability will then be quantified, in order to evaluate the importance of integrating speciation in LCA. At present, preliminary characterization factors for terrestrial ecotoxicity are obtained using commercially available speciation models (WHAM 6.0 and MINEQL+) and IMPACT 2002 and confirm the need to develop a speciation model specific to soils.

**776 Characterizing the Spatial Heterogeneity of Hazardous Air Pollutant Emissions from On-road Motor Vehicles** A. Lobscheid, M. Spears, Lawrence Berkeley National Laboratory; T. McKone, Univ of California, Berkeley, Univ of California and Lawrence Berkeley National Laboratory, Univ of California, Univ of California and Lawrence Berkeley National Laboratory. On-road motor vehicles (MVs) in the United States (US) emit approximately  $10^9$  kg per year of conserved (non-reactive and non-depositing) hazardous air pollutants (HAPs) to the atmosphere. Within life-cycle impact assessment (LCIA), there is a need to spatially resolve the population impacts associated with these HAP emissions. Primarily, this need centers around identifying the level of resolution that adequately captures the spatial variability of emissions and resulting health impacts associated with atmospheric emissions. In order to explore this spatial variability, we estimate population impacts using the intake fraction (iF) – the ratio of the chemical mass taken in by the population to the mass emitted. The population iF [ppm] is estimated from the on-road MV source-receptor relationships derived from the US Environmental Protection Agency's AERMOD model, which was employed in the latest (2005) National Air Toxics Assessment (NATA). AERMOD is a steady-state plume model that incorporates air dispersion to characterize atmospheric concentrations of MV emissions. AERMOD provides source-receptor (S-R) relationships ( $X/Q$ ), in  $\text{mg/m}^3$  per ton/y emitted, for 64,600 census tracts in the conterminous US. The mean S-R-based iF across these census tracts is 8.5 ppm, the median is 3.8 ppm, and the inter-quartile range (IQR) is 0.8 to 8.9 ppm. When census

tracts are aggregated at the county-level, and subject to a unit emission, the mean population iF is 1.3 ppm, the median is 0.4 ppm, and the IQR is 0.13 to 1.2 ppm. We explore alternative methods, or “approximators”, that can be easily integrated within an LCA to characterize spatial variability of health impacts. Examples include a box-model approach adjusted for chemical persistence to estimate county-level iF, and correlations between the population density of the source region (county) and the iF obtained from the AER-MOD derived S-R ( $R^2 \sim 0.8$ ). The population iF estimates can be integrated with HAP emissions to characterize health impacts of transportation fuels. This is particularly useful in LCIA methods that relate health impacts from second and third generation cellulosic biofuels, with conventional gasoline.

**777 Assessing Drinking Water Intake of Aluminum and Arsenic Emissions** A. Henderson, Univ of Michigan, Environmental Health Science, School of Public Health, Univ of Michigan, School of Public Health; O. Jolliet, Univ of Michigan, School of Public Health. In assessment of the fate, transport, and ultimate exposure of metals to humans, correctly accounting for long-term groundwater emissions, e.g., from a landfill, has been challenging. This research offers a new perspective on human exposure to anthropogenic metal emissions to ground and surface water. Existing models, such as the Ecoinvent LCA database (Frischknecht et al. 2004) have assumed 100% of landfill emissions reach surface water. To accurately reflect geochemical and hydrologic processes, this conservative assumption can be reassessed. Other sources of metals, such as mining of aluminum ore, may generate emissions directly to surface water. Models of metals in surface water (e.g., Gandhi et al. (2010)) likewise have potential for improvement; e.g., via accounting for the possibility of precipitation. A multi-component model was developed to assess the total intake by humans of emissions to landfills (for aluminum) and directly to surface water (for arsenic from aluminum ore). For landfill emissions, a kinetic precipitation model was developed to estimate transfer to surface water. In surface water, the geochemical model PHREEQC (Parkhurst 1995), including an implementation of WHAM VI (Tipping 1998), was used to estimate speciation, as well as the potential for precipitation, sorption, and complexation, while hydrological data at the continental level provided estimates of continental flows and residence times. Information at the national level on drinking water source and treatment were aggregated to the continent scale and included in the model, thus providing estimates of the population fraction drinking treated and untreated ground and surface waters. Finally, speciation was used to assess bioavailability of ingested aluminum to humans. The model shows lower estimates of transport from source to receptor than previous studies, which lacked a precipitation module. Uncertainty in input data and modeling assumptions, which will be discussed, allow the model to be applied to large-scale systems as a comparative tool. For example, the ingested intake fraction for aluminum emissions to landfills is lower than previous models by a factor of  $1 \times 10^{-6}$  or more, depending on the location. The continent of emission, via its hydrology and the fraction of treated, untreated drinking water, plays an important role.

**778 Life Cycle Assessment and the Work Environment: Case Study in Municipal Solid Waste End-of-Life Management Methods** K. Scanlon, The George Washington Univ, Dept of Environmental and Occupational Health; R. Francis, The George Washington Univ, Dept of Engineering Management and Systems Engineering; S. Lloyd, Concurrent Technologies Corporation. Impacts to occupational health are often neglected in life cycle assessment due to data limitations, methodological limitations, and lack of professional subject matter expertise. However, decisions made in the absence of quantified occupational health data may result in sub-optimization, where one portion of a system may be improved ignoring the effects on another portion of the system leading to unintended consequences. This presentation will demonstrate how publicly-available work-related fatal and nonfatal injury and illness data can be used to characterize impacts to occupational health across a product life cycle. It will introduce a new life cycle impact assessment (LCIA) method for characterizing these impacts. Also, this presentation will describe the method used to develop work environment characterization factors and a new damage impact category indicator, the work environment disability adjusted life year. The findings from a case study in municipal solid waste end-of-life management methods will be presented. The case study will demonstrate the application of the work environment LCIA method and highlight its strengths and shortcomings. The presentation will end with suggestions for future case studies to qualify this new impact assessment method.

**779 The Unnatural Nature of Communicating Risks in Natural Disasters** M.Y. Lichtveld, Tulane Univ SPHTM, Environmental Health Sciences; J.K. Wickliffe, Tulane Univ, Environmental Health Sciences; L. Bondi-Marschner, C. Mundorf, Tulane Univ SPHTM, Environmental Health Sciences. The Gulf of Mexico Oil spill, the Tsunami-induced nuclear reactor disaster in Japan, and the Morganza flooding in Louisiana demonstrate the myriad of problems in effective risk communication. The lack of effective, action-driven risk- and perhaps more appropriate crisis communication- not only profoundly affects community protection but also science, policy, and practice. Knowledge gaps regarding environmental and toxicological consequences of natural disasters can pose formidable challenges for risk assessors and toxicologists balancing uncertainty and responding to community concerns. This is compounded by the interconnectedness and fluid nature of technological and natural disasters, e.g., the impact of future hurricanes on the Gulf of Mexico Oil spill. In Louisiana, Hurricane Katrina, the Oil spill, and the Morganza flooding provide a unique triad of case studies: First, the qualifications of the messenger go much beyond risk communication or discipline-specific expertise; rather, the successful risk communicator is often homegrown, capable of harmonizing scientific uncertainty and community responsiveness. Secondly, the message has to withstand scientific scrutiny, but also that of victims. Thirdly- and most importantly- the timing, “longevity”, and actionable nature of the message are all prerequisites for success. The increasingly powerful role of social media affects the signal transmission speed and multiple stakeholder agendas decrease the signal-to-noise ratio rather than improving the signal. The overarching lesson not learned is that of context and precedent. For example, many communities affected by the oil spill and the Morganza flooding still remain in or were just emerging from a Post-Katrina recovery state. Preliminary findings from disaster research conducted as part of the SECURE Consortium funded by NIH show that disaster recovery is influenced by a series of experiences including loss of livelihood; direct physical adverse health effects; community stress; fear of the unknown; unfounded fear of the known; indicators of family stress; mixed messages and an influx of non-local personnel. Communicating in this context profoundly affects risk perception. Similarly, previous negative risk communication experiences directly impact trust and message actionability. This presentation will present a culturally competent framework for crisis communication in complex interconnected natural and technological disasters.

**780 Fishing for Answers in the Willamette River: The Mystery of the Newberg Pool** L. Jenkins, Oregon State Univ, Dept of Environmental & Molecular Toxicology. In the early 1990's news reports of a high prevalence of skeletal deformities in fish located in a slow moving reach of the Willamette River near Newberg was concerning Oregonians. Were the fish deformed by unseen poisons that could also endanger human health? Local concern was focused on a new water treatment plant just downstream at Wilsonville. People refused to drink the water. What was in the Newberg Pool? Following additional studies commissioned by the Oregon Dept of Environmental Quality that confirmed the continued high prevalence of fish deformities but were unable to identify a cause, in 2001 the legislature called on Oregon State Univ to find out what was causing fish deformities in the Newberg Pool. A multidisciplinary team was assembled and the mystery of nature was transformed into a researchable question. The team included researchers with expertise in aquatic toxicology, ecotoxicology, environmental analytical chemistry, and fish biology and systematics, fish disease and parasitology, and human health risk assessment and risk communication. The research goal was not only to solve the mystery and report back to the legislature, but also provide a scientific basis for public outreach and risk communication. This case study will present an example of outcome-driven multidisciplinary research and outreach designed to provide risk information useful to individual decision-making and public policy, and to promote transparency and dialogue about risks associated with exposure to environmental agents.

**781 Lessons in Communication: Emergency Response, CSX Transportation, Brooks, Kentucky** P. Kurzanski, CSX Transportation; J.R. Clarkson, Arcadis US, Inc., Senior Vice President, ENVIRON, ENVIRON. On January 16, 2007, at 8:51 a.m., a CSX train en route to Louisville from Birmingham derailed in Brooks, Kentucky. The train consisted of 4 locomotives and 80 cars of which 41 were loaded and 39 were empty. A total of 25 cars derailed of which 12 were carrying hazardous materials. The potential for environmental impacts to the following: air, surface water, sediments,



soil, groundwater, seeps, and residential drinking water (private wells and municipal water supply) were evaluated. Materials from the derailed cars flowed into a storm water management ditch along the railroad track through unnamed tributaries of Floyds Fork, into Floyds Fork (stream) with potential to ultimately reach the Salt River, approximately 3.5 miles downstream. Homes along the creek used the water for recreation, livestock and pets. The creeks, stream, and river were used by aquatic and terrestrial flora and fauna. At the confluence of the Floyds Fork and Salt River was an intake for a municipal water supply. Residents in 15 homes near the train tracks were temporarily displaced until clean up and restoration were completed. Air, surface and subsurface soils and groundwater/seeps around the derailment site were also potentially impacted. A number of constituents of concern were evaluated for human and ecological health depending upon the media and circumstances surrounding the response actions, but the primary constituents associated with the train were 1,3-butadiene, methyl ethyl ketone and cyclohexane. The strategic management of this incident by CSX was excellent and serves as a great model for lessons learned. Communication was a critical component to this successful management. This presentation will highlight strategic risk communication lessons learned during the emergency, post-emergency, and restoration phases of the transportation incident. The oversight by federal, state, and local regulatory agencies, the media, and the public was intense and presented many risk communication challenges that continued to change throughout the three phases of the incident. Communication of potential risks to human and ecological health was critical to the overall successful management and resolution of this incident.

**782 Use of Risk Assessment to Demonstrate Benefits to Local Stakeholders of Regional Action to Decrease Greenhouse Gas Emissions** R.N. Hull, D.W. Bryant, Intrinsik Environmental Sciences Inc.; E. Sigal, Intrinsik Environmental Science Inc. A risk assessment was conducted as part of a Climate Change Project that had the goal of decreasing Egypt's greenhouse gas emissions, focusing on a cluster of brick factories. While greenhouse gas reduction is an important national and global goal, it is relatively meaningless to those most directly affected by the project (i.e., the brick factory owners, workers, residents of the surrounding communities, and government officials). Brick factories in the Arab Abou Saed region of Helwan, Egypt have been burning heavy fuel oils as the primary fuel source since the mid-1980s. Poorly controlled heavy fuel oil combustion results in the release of many chemicals and particulate matter are released to the surrounding environment. Conversion of the brick factories from the use of heavy oil to natural gas will greatly reduce both the quantity and type of pollutants emitted from brick factories. Risk assessment was used to provide a systematic, scientific means for quantifying, comparing, and prioritizing risks associated with this conversion. The risk assessment addressed the range of local health and environmental risks associated with burning heavy oil, and the potential benefits (risk reduction) after conversion of an increasing percentage of the factories to natural gas. A detailed review of epidemiological data on health benefits of pollution reduction also was included to assist in communication of the benefits of the conversion. The effective communication of the meaning of the potential for improved air quality and reduced risks to the diverse group of stakeholders was crucial to the success of the pilot project and to the potential for future conversion of additional factories. Successful communication of risk assessment results garnered the full support of all stakeholders for the pilot project, which is expected to result in several benefits including improvements in health, the local economy, and reduction of greenhouse gas emissions.

**783 Use of HHRA and SLERA Results in a Communication Program to Support Siting a Resource Recovery Facility in Puerto Rico** B.J. Locey, ARCADIS, Risk Assessment and Environmental Services; J. Conklin, ARCADIS, RAES; K. Hallinger, ARCADIS; J. Hanisch, ARCADIS, EMPC; J. Smith, ARCADIS, RAES; M. Green, Energy Answers International; A. Molinare, Univ of Puerto Rico and Universidad Metropolitana Cupey Campus, Univ of Puerto Rico, Río Piedras Campus. Permit and Regulations Program, Special Education Division. Universidad Metropolitana, Cupey Campus. Graduate School of Environmental Affairs. Human health (HHRA) and screening-level ecological (SLERA) risk assessments were prepared to evaluate emissions from a proposed resource recovery facility in Puerto Rico. The assessments were part of the Environmental Impact Statement (EIS) submitted and approved by the Puerto Rico Environmental Quality Board in 2010. The results of these risk assessments are being used

to support an on-going communication program aimed at providing information about the proposed facility and proactively addressing local residents' concerns. This presentation will provide an overview of the approach used to evaluate potential risks and hazards to human and ecological receptors and will focus on how the results have been used in the communication program. The overview will briefly present the approach used to estimate constituent emissions, complete the air dispersion and deposition modeling, identify geographic areas and populations of interest for both the HHRA and SLERA, perform fate and transport calculations to estimate constituent concentrations in the environment, and evaluate potential risks and hazards. Some challenges and solutions to obtaining area-specific information needed for the assessments will be discussed. The communication program will be described, highlighting the approaches used to address local residents' concerns, navigate the political climate, and accommodate mixed agendas of various groups. The project team has used a proactive, multi-pronged approach to identify specific concerns of local groups and has addressed them in a way that is tailored to their needs. The landscape surrounding the proposed facility includes an urban center, agricultural fields, dairy farms, a drinking water reservoir, and water bodies that are routinely fished by locals. Additional information and clarification have been presented to local residents in meetings with individuals, in town meetings, through radio broadcasts, and in special forums that address concerns about specific chemicals (e.g., dioxins, lead, mercury), health concerns, and the approach used to evaluate the potential impacts of the facility. Unique challenges presented by the local political climate will also be discussed.

**784 Risk Communication to Multiple Audiences: A Case Study** S.L. Sager, J.S. Beckner, ARCADIS. A former industrial site was dismantled and the land deeded to the county in North Carolina. The County then redeveloped a portion of the property as a year-round school. Later, when the County considered redeveloping other portions of the property, a limited soil investigation was conducted. The results of the investigation indicated that several metals and organic compounds were present in soil samples. Based on the sampling results, it was necessary to collect additional soil and groundwater samples and a communication plan developed. The results of the additional site investigation indicated that no immediate threat to the children existed. Nonetheless, since some of the soil concentrations were above levels protective of residential exposures and were in student use areas, the decision was made by the stakeholders to reduce soil constituent levels to meet residential standards. As a year-round school, there was a limited window in which the soil removal could occur and not disrupt activities at the school. Thus, there was a lag time between the decision to remove soil and implementation. This set the stage for a risk communication program to inform staff and parents about the findings and removal strategy. The program involved dissemination of the sampling results to the school community and addressed the community's questions and concerns. The initial element in the risk communication program was to discuss with school staff the ways to minimize exposure of the students and staff to constituents in soil. Since the goal was to minimize exposure of the children and still allow use of the playground, once the children returned from the playground, they were directed to sinks for hand washing immediately upon entering the building. Use of other open areas of the property for picnics and gardens was suspended until remediation was complete. Finally, a series of meetings with parents and staff about the studies, results, and remedial strategy were held to communicate the information to the school community and garner their support for the remedial strategy ultimately identified for the parcel. One of the key concerns of the stakeholders was the loss of the use of the playground prior to the end of school. This paper will present the approach taken to characterize and communicate the results of the evaluation.

**785 A Comprehensive Evaluation of Risk to Human Health and Ecological Receptors in a PCB-contaminated Floodplain** M.H. Henning, A.R. Glessner, A.L. Fogg, D. Pelletier, C. Stubbs, ENVIRON International Corporation; J. Johnson, Bridgestone Americas, Inc. During historical flood events, PCB-contaminated sediment from Stony Creek (Noblesville, IN (USA)) was deposited on both the undeveloped floodplain and residentially developed floodplain surrounding the creek. Risks to human health and ecological receptors from PCBs were evaluated in both the undeveloped and residentially developed areas. The human health risk assessments for the residential properties were conducted using a streamlined, spatially-explicit approach that compared pre- and post-remediation exposure point concentrations to a site-specific risk based closure level. Residents were informed of

the pre-remediation risk assessment conclusions and participated in the selection of remedial actions on their property. In addition, human health and ecological risk assessments were performed on the undeveloped floodplain which is currently under a conservation easement. Risk conclusions were communicated with the property owner, land trust manager, and City of Noblesville. Successful communication with all stakeholders, including the USEPA, led to the Final Decision being issued by the USEPA in December 2010. This presentation will discuss the risk assessments conducted in the floodplains and how successful communication among all parties resulted in a Final Decision.

**786 Engaging Stakeholders in Environmental Management Decision Using Formal Decision-Analytical Tools** L. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center; Z. Collier, M. Bates, US Army Corps of Engineers, Engineer Research & Development Center. Stakeholders often play a major role in decision making involving environmental risks. Traditionally, project teams have informed stakeholders on the course of action and their values and opinions have been integrated qualitatively in an ad-hoc manner, based on verbal or written feedback elicited from stakeholders. This is not ideal due to the large degree of information lost and the tendency of the loudest or most insistent opinion to be the only one heard. Furthermore, a qualitative approach to stakeholder engagement does not allow for the expression of value judgments or for dealing with tradeoffs. Lastly, stakeholders can have a great variety of differing viewpoints that needs to be balanced and integrated. Multi-criteria decision analysis (MCDA) offers a means to integrate value judgments into complex decision-making processes. By visualizing individual and often conflicting stakeholder views, it can also serve as a tool to resolve conflicts which would normally prevent action in an important yet contested area. By quantitatively accounting for stakeholder opinions and concerns, their ideas can be integrated into decision-making in a real and transparent way so that their views are accounted for. This presentation will present multiple examples in which stakeholders' opinions have been integrated into complex environmental decision-making processes. Examples will be presented which show the integration of stakeholders from local communities engaged in management of contaminated sites as well as federal agencies deciding on the best course of action. Integrated risk management and decision analysis tools to resolve stakeholder conflicts will be specifically discussed.

**MP001 QSAR and Jumping Fragment for the Assessment of Classification in Ecotoxicology** R. Bureau, S. Lozano, CERMN, Univ of Caen; B. Cuissart, G. Poezevara, GREYC, Univ of Caen; L. Alban, CERMN, Univ of Caen; B. Cremilleux, GREYC, Univ of Caen. A new approach combining QSAR analysis and jumping fragments will be presented. Two objectives could be reached. The first one concerns an estimation of the potential classification of a derivative in ecotoxicology. The second objective is the possibility to get more information about the potential mode of action (MOA) associated to a derivative. The QSAR equations were mainly based on the integration of parameters in relation with hydrophobic, steric or electronic characteristics of chemicals. They were designed to estimate the toxicity in relation with a non specific MOA like baseline narcosis, polar narcosis, ester narcosis and some classes of reactive chemicals (the toxicity of high reactive chemicals is underestimated). In these global equations, polar and non polar compounds are handled in the same models. The second point of this study concerns the potential application of jumping fragments for C&L. A recent data mining algorithm was experimented enabling an automatic extraction of substructures which appear frequently in one class (H400) and never appear in another class (H402). Such a substructure was named a jumping fragment. The overall objective of this study was to analyse the potential of these approaches starting from a random set of derivatives for the estimation of their potential classifications.

**MP002 Estimating Abraham's Hydrogen Bonding Acidity Parameter Using Quantum Chemistry** D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering; O. Rahaman, D.J. Doren, Univ of Delaware, Dept of Chemistry. Linear solvation free energy polyparameter models are widely used to estimate partitioning coefficients between water, air, and environmental phases such as organic carbon and organism lipid. Applying these models to a new chemical requires the chemical's parameters that describe the electrostatic, hydrogen bonding, and dispersion interactions. These can be either measured using various methods such as water-solvent partitioning or HPLC column retention times, or estimated using QSAR techniques. We present a new method for estimating the hydrogen bond donation parameter (the H bond acidity) based on quantum chemically computed gas phase enthalpy of binding to various hydrogen bond accepting molecules (H-bond bases). The method is calibrated using acidity constants that are derived from direct experimental determination between sets of H-bond acids and bases. The accuracy and predictive ability will be presented. In particular the treatment of bidentate binding – where the acid can act both as an acid and a base when interacting with the base – will be discussed.

**MP003 Comparison of Theoretical and Experimental Models for Characterizing Solvent Properties Using Reversed Phase Liquid Chromatography (RPLC)** L. Tao, Karolinska Institute, Institute of Environmental Medicine (IMM), Unit of Work Environmental Toxicology, Institute of Environmental Medicine (IMM); I. Nicholls, Dept of Biochemistry & Organic Chemistry Laboratory, Uppsala Univ; School of Natural Sciences, Linnaeus Univ; T. Oberg, Linnaeus Univ, School of Natural Sciences. The properties of solvents are critical for determining the behaviour and interaction of molecular structure. We develop quantitative structure retention relationship (QSRR) models to characterize solvent properties in reversed phase liquid chromatography (RPLC). We selected solvents using principal component analysis (PCA) approach to ensure a maximum spread in the solvent properties, and used analyte, the herbicide atrazine, in a system derived from nine organic solutes (methanol, ethanol, 1-propanol, 2-propanol, tetrahydrofuran, acetone, acetonitrile, 1,4-dioxane, 2-methoxyethanol) at four different concentration and using three chromatographic columns (C8, C12, C18). Multiple linear regression (MLR) and partial least squares regression (PLSR) were used as statistical approaches. The similarity and difference between modified global linear solvation energy relationship (LSER), semi-empirical, and theoretical molecular models were demonstrated, and the similarity and difference between experimental and theoretical model approaches were compared and discussed. The results showed that the retention factors of new mobile phase can be successfully predicted using both of experimental and theoretical approaches, the prediction of retention phenomena can reduce cost and time consuming in developing chromatographic methods and providing a better understanding of solute-solvent interactions. The results were in accordance with the free energy transfer that sum up van der Waals and polar interactions.

**MP004 Using QSPRs, Chemical Fate Models and the Chemical Partitioning Space to Investigate the Fate of Chemical Mixtures with Very High Number of Components** A. Gawor, F. Wania, Univ of Toronto, Dept of Chemistry. A number of environmentally relevant substances are chemical mixtures with very high numbers of constituents. This includes industrial substances such as the chlorinated paraffins, unintended by-products such as mixed halogenated dibenzo-*p*-dioxins and -furans, and pesticides such as toxaphene. The mixture constituents are structurally related, yet differ in terms of the degree of halogenation and the substitution patterns. The sheer number of mixture constituents, which can exceed many thousand individual molecules, and the wide range of fate-relevant properties of these molecules make the environmental fate, exposure, and risk assessment of such substances particularly challenging. Even the selection of representative mixture constituents that might be able to serve in the assessment of the entire mixture is not trivial. Here we outline an approach based on the structural identification of mixture constituents, the estimation of their fate relevant properties using different quantitative structure property relationships (QSPRs), and the placement of the mixture constituents on a variety of chemical space maps. The latter allows for a fast, visual assessment of the fate and behaviour in the environment of the mixture and its constituents, including their phase distribution in water, soil and atmosphere, as well as their bioaccumulation and long range transport potential. In particular, the maps reveal how environmental fate changes with, and depends on, structural characteristics such as degree and type of halogenation, or chain length. It further allows for an informed selection of a small number of constituents that are representative of the entire mixture, e.g., for more detailed assessments or the experimental confirmation of the QSPR-predicted fate-relevant properties.

**MP005 Application of Ionic Liquid 1-octyl-4-methylimidazolium Hexafluorophosphate as a Recyclable Solvent for Treatment of Mercury(II) Contaminated Water** S. Chen, Beijing Univ of Technology; X.W. Wang, Beijing Univ of Technology; Research Center for Eco-Environmental Sciences, Chinese Academy of Science; J. Liu, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Room temperature ionic liquid 1-octyl-4-methylimidazolium hexafluorophosphate [C<sub>8</sub>mim]PF<sub>6</sub> was taken as extraction agent for liquid/liquid extraction of Hg<sup>2+</sup> in water. After the extraction, Hg<sup>2+</sup> can be reduced and removal from [C<sub>8</sub>mim]PF<sub>6</sub> using formic acid through being heated at 50 °C effectively. The concentration of mercury in water and ionic liquid was detected by cold vapor atomic absorption spectrometry, respectively. The results show that, the [C<sub>8</sub>mim]PF<sub>6</sub> is a effective extractor for the extraction of Hg<sup>2+</sup> in water. When the phase ratio of aqueous phase to ionic liquid phase is not higher than 10:1, the [C<sub>8</sub>mim]PF<sub>6</sub> shows high extraction efficiency over 90 % for 1 mg/L Hg<sup>2+</sup> solution at stirring 0.5 hour under 50 °C. With the increase of the phase ratio, the extraction efficiency decrease. But the extraction efficiency remains close to 60 % when the phase ratio is 25:1. On the other hand, formic acid can be highly effective reduction and removal of Hg<sup>2+</sup> in the ionic liquid, and the reduction process will not produce a serious damage the properties of the ionic liquid due to its relatively low temperature and short time. Acknowledgements This study was supported by the Beijing Natural Science Foundation (project number: 8092008), and by the Air Pollution Control and Innovative Technical Team (number: PHR201007105)

**MP006 A Comparative Health and Environmental Hazard Assessment for Antimony Trioxide and Halogenated Flame Retardants** C. Kelly, AMEC Geomatrix, Inc.; C. Mackay, AMEC. One of the new inorganic alternative flame retardants finding use today is antimony trioxide (Sb<sub>2</sub>O<sub>3</sub>). Sb<sub>2</sub>O<sub>3</sub> is being marketed to replace the organic flame retardants in the textile industry. Here we will present the results of a comparative health and environmental hazard assessment for antimony trioxide (Sb<sub>2</sub>O<sub>3</sub>) and halogenated flame retardants. Our research on antimony trioxide indicates that there is little potential for human exposure in various clothing and in bedding materials. We will also highlight evidence that antimony trioxide possesses very low environmental toxicity and does not bioaccumulate in humans or in aquatic or terrestrial food chains. This is in contrast to other flame retardant agents such as the brominated biphenyls, chlorinated paraffins, and perfluoro compounds. These compounds accumulate in human fat tissue, manifest specific toxicities (including carcinogenicity), and are persistent and biomagnify in the environment.



**MP007 Contamination Status and Accumulation Features of Brominated Flame Retardants in Raptors from Japan** T. Isobe, Ehime Univ, Senior Research Fellow Center, Ehime Univ, Senior Research Fellow; R. Hashikawa, Ehime Univ, Center for Marine Environmental Studies; S.H. Hirata, Tottori Univ, Dept of Regional Environment; K. Nomiyama, Ehime Univ, Center for Marine Environmental Studies (CMES); H. Mizukawa, Ehime Univ, Center for Marine Environmental Studies; T. Hayashi, Tochigi Prefectural Museum; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies. Environmental contamination by brominated flame retardants (BFRs), especially by polybrominated diethyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs), are of public concern due to their persistence, bioaccumulative nature, and possible adverse effects on humans and wildlife. Both chemicals are used as additive flame retardants in a wide variety of commercial and household products to reduce their flammability. As a result, they are ubiquitous pollutants in the environment and biota and accumulate in higher trophic level animals. Raptors, a group of predatory birds, feeding high in the food web and accumulate persistent lipophilic contaminants. Although there are some reports on contamination by organochlorines in predatory birds, information on BFRs are still limited. The present study investigates the contamination status and accumulation features of PBDEs, HBCDs and PCBs in pectoral muscle of raptors from Tochigi Prefecture, Japan. Forty two individuals belonging to nine species including goshawk (*Accipiter gentilis*,  $n=19$ ), owl (*Strix uralensis*,  $n=7$ ), European sparrowhawk (*Accipiter nisus*,  $n=5$ ), black kite (*Milvus migrans*,  $n=4$ ), common buzzard (*Buteo buteo*,  $n=2$ ), grey-faced buzzard-eagle (*Butastur indicus*,  $n=2$ ), peregrine falcon (*Falco peregrinus*,  $n=1$ ), sparrowhawk (*Accipiter gularis*,  $n=1$ ), mountain hawk eagle (*Spizaetus nipalensis*,  $n=1$ ), were collected. Analysis of BFRs (PBDEs and HBCDs) and PCBs were carried out following the procedures described elsewhere. PCBs and PBDEs were determined using GC-MS, whereas HBCDs were quantified by LC-MS/MS. PBDEs and PCBs were detected in all the specimens, and HBCDs were also found in most of the samples (36/42), indicating ubiquitous contamination of terrestrial biota by these organohalogen compounds. PBDEs concentrations ranged from 10 to 46,000 ng/g lipid, which were comparable to a previous report which found 32,500 ng/g lipid of PBDEs in sparrowhawk from Belgium. Concentration range of HBCDs was < 0.005 – 5,700 ng/g lipid and was greater than that in Guillemot (*Uria aalge*) from the Baltic Sea (mean: 65 ng/g lipid). Since HBCDs are extensively used and market demand is still growing in Japan, continuous monitoring surveys of terrestrial ecosystems are warranted. On the other hand, PCBs concentrations (230 – 190,000 ng/g lipid) were one to two orders of magnitude higher than BFRs, suggesting high-volume production in the past, leakage from stockpiles and persistence of these compounds.

**MP008 Depositional Trends of Halogenated Flame Retardants to Remote Lake Sediments, Lake Simcoe, and Lake Ontario in the Great Lakes Region** L. Shen, Brock Univ; E. Reiner, Ontario Ministry of the Environment; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; C. Marvin, Environment Canada; K. MacPherson, T. Kolic, Ontario Ministry of the Environment; D. Muir, Environment Canada; G. Tomy, Fisheries and Oceans Canada; I. Brindle, Brock Univ. Halogenated flame retardants (HFRs) have been used as additive flame retardants in a wide variety of applications including electronics, building materials, carpeting, and textiles, and are detected in the environment as a result of their use. Historical trends of current and past-use chemicals of concern in the Great Lakes region are necessary to evaluate policies for virtual elimination of priority pollutants, and for establishing baseline information to assess new regulatory actions within the basin and globally. In this study, sediment cores were obtained from remote lakes in or near the Great Lakes region from Siskiwit Lake on Isle Royale in L. Superior, Experimental Lakes Area in northwestern Ontario, and Plastic Lake in south-central Ontario. Concentration trends and sedimentation fluxes were compared for several HFRs in the remote cores to those collected from L. Simcoe and L. Ontario, which are both impacted by industrial and urban inputs. HFRs included were Dechlorane Plus (DP), Dechlorane 602 and 603 (Dec 602, Dec 603; all chlorinated FRs) and Dechlorane 604 (Dec 604; a brominated and chlorinated FR), and the brominated FRs decabromodiphenylethane (DBDPE), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), octabromotrimethylphenyl-indane (OBIND), bis(2-ethyl-1-hexyl)tetra bromophthalate (BEHTBP), and BDE-209. In Lake Ontario, Dec 602, 604, and DP concentrations increased in sediments between the mid-1960s and mid-1970s, approximately 10 years after Mirex, for which they

are replacements. Maximum concentrations of Dec 602 and DP occurred in the early 1980s, and Dec 604 in the mid-1980s followed by declines which have not been as rapid as for Mirex. The dechlorane compounds were detected in remote lakes and Lake Simcoe, but at lower concentrations than in L. Ontario. Declines in L. Simcoe were smaller than in L. Ontario, and recent sediments had the highest concentrations in the remote cores for DP and Dec 602. Several alternative brominated flame retardants, including BTBPE, BEHTBP, OBIND, and DBDPE, were detected in remote and impacted lake sediments. Concentrations were lowest in the remote cores, but the highest concentrations were observed in either the surface or top 2 cm sediment slices. Sedimentation fluxes will be calculated based on dated cores and compared between lakes to estimate the role of atmospheric deposition.

**MP009 GPS-based Biotelemetry Meets Ecotoxicology: A Novel Approach for Understanding the Exposure of Avian Species to Organohalogen Contaminants** M. Gentes, Environmental Toxicology Research Centre (TOXEN), Dept of Biological Sciences, Univ of Quebec in Montreal; J.F. Giroux, Univ of Quebec in Montreal, Behavior and Animal Ecology Research Group (GRECA), Dept of Biological Sciences; R. Letcher, Environment Canada, National Wildlife Research Centre, Wildlife and Landscape Science Directorate, Science and Technology Branch, Carleton Univ; M. Patenaude-Monette, Univ of Quebec in Montreal, Behavior and Animal Ecology Research Group (GRECA), Dept of Biological Sciences; J. Verreault, Univ of Quebec in Montreal, Environmental Toxicology Research Centre (TOXEN), Dept of Biological Sciences. A large suite of non-reactive flame retardants (FRs) are routinely added to consumer products to comply with fire safety standards. The now largely restricted polybrominated diphenyl ethers (PBDEs) are ubiquitous in nearly all biotic and abiotic media worldwide, and their unregulated replacement products [e.g., decabromodiphenyl ethane (DBDPE), BTBPE (1,2-bis(2,4,6-tribromophenoxy) ethane), pentabromoethylbenzene (PBEB), etc.] are currently being detected in tissues and eggs of avian species (e.g., raptors, gulls). However, analysis of organohalogen contaminant data (including PBDE/FR) collected as part of avian biomonitoring programs is often hampered by the lack of characterization of bird's feeding ecology. These knowledge gaps can lead to critical misinterpretations of exposure sources to organohalogen contaminants. In Canada, studies investigating FR contamination in avian species breeding in the highly industrialized and polluted areas of the St-Lawrence River, a known FR contamination hotspot in Eastern Canada, are particularly scant. PBDEs and some unregulated FRs have been detected in gull eggs (herring and ring-billed), but sources of exposure have never been specifically investigated. Using a novel approach based on GPS-tracking of ring-billed gulls (RBGUs) equipped with miniature dataloggers, movements of individuals have been precisely reconstructed and mapped with ArcGIS. The links between habitat-use (i.e., exposure to suspected sources of FRs such as landfills, wastewater plant, industrial parks, etc.) and contamination profiles of individual gulls (blood plasma and liver) were investigated. Preliminary results confirmed the presence of major emerging FRs (e.g., DBDPE, BTBPE, anti-Dechlorane plus (DP), etc.) in RBGU's blood, and concentrations vary greatly both among individuals and for different compounds. For instance, the highest mean plasma concentration determined for PBDEs was 7.43 ng/g ww, while that of anti-DP was 0.33 ng/g ww. Analyses of remaining plasma and liver samples for PBDE/FR are in progress. Preliminary inspection of GPS tracks revealed important space-use diversity, i.e., some individuals foraged mostly in agricultural or riparian areas, while others visited human impacted areas (landfills, urban cores, etc.). Data analysis is ongoing and detailed results will be presented at the conference.

**MP010 Metabolism of Organophosphate Flame Retardants by Human Liver Microsomes and Porcine Esterase** E.M. Cooper, Duke Univ, NSOE; H.M. Stapleton, Duke Univ, Nicholas School of the Environment. The organophosphates (OPs) tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenyl phosphate (TPP) are common flame retardants (FRs) present in automobiles and many household products. Levels of TDCPP and TPP measured in indoor environments suggest that chronic exposure is occurring for the general population. However, no studies have examined the risks to human health, and knowledge of the human body burdens and metabolism of these FRs is poorly understood. We investigated TDCPP and TPP metabolism in vitro with pooled human liver microsomes and porcine esterase using solid phase extraction and liquid chromatography tandem mass spectrometry. The microsomes metabolized TDCPP and TPP to their respective diester products, bis (1,3-dichloro-2-propyl) phosphate (BDCPP)

and diphenyl phosphate (DPP), as primary metabolites. Rates of transformation were  $95.8 \pm 2.7$  pmol/mg protein/min for TDCPP and  $78.8 \pm 2.7$  pmol/mg protein/min for TPP. The esterase biotransformed 50% of 1.3 nmoles of TDCPP and 34% of 0.6 nmoles of TPP. DPP accounted for 68% of the mass of TPP lost after incubation with the esterase. However, BDCPP observed was less than 1% of initial TDCPP added, suggesting that BDCPP was further metabolized, and/or that other metabolites were formed that were not monitored. Further experiments will measure the kinetics of their metabolism, explore differences in the extent of the metabolism between the two FRs, the enzyme system involved, and examine tissue specific differences in metabolism.

**MP011 Occurrence of Organophosphate Esters in Songhua River and a Municipal Wastewater Treatment Plant in China** J. Liu, X. Wang, Y. Yin, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Organophosphate esters (OPEs) are widely used as flame retardants and are now broadly present in the domestic and outdoor environment. In this study a high throughput LC-MS/MS method was developed for the determination of twelve OPEs including trimethyl phosphate (TMP), triethyl phosphate (TEP), tris(2-chloroethyl) phosphate (TCEP), tri-n-propyl phosphate (TnPP), tris(2-chloroisopropyl) phosphate (TCPP), tris(2-chloro-1-(chloromethyl)ethyl) phosphate (TDCP), triphenyl phosphate (TPHP), tributyl phosphate (TnBP), tris-(2-butoxyethyl) phosphate (TBEP), tri-3-cresyl phosphate (TCrP), 2-ethylhexyl diphenyl phosphate (EHDPP) and tris(2-ethylhexyl) phosphate (TEHP). Analytes were concentrated by solid phase extraction and then determined by ultra-high performance liquid chromatography (Acquity UPLC, Waters, US) equipped with a triple quadrupole mass spectrometer (TSQ Quantum Access, Thermo Scientific, US). The developed new method is capable of baseline separating 11 of the 12 studied OPEs within 11 min with LOQs ranged from 0.5 to 20 ng/L and relative standard deviations in the range of 2-10%. For both reagent water and river water, the spiked recoveries of OPEs ranged from 69-110%, except for the very polar and volatile trimethyl phosphate that has recovery below 10%. This developed procedure was applied to study the OPE contamination status in China for the first time. Water samples collected from 17 sites along the Second Songhua River and Songhua River, which is the main river in the northeast part of China and has a total length of 2214.3 km, were analyzed for the first time. All the target twelve OPEs were detected with total concentrations of OPEs around 1  $\mu\text{g/L}$  in the river waters collected from each sites. The concentration of individual OPEs ranged from 0.85 ng/L for TnPP to 3700 ng/L for TCEP. In all the 17 water samples TCEP (38-3700 ng/L) and TnBP (87-870 ng/L) were the major contaminants, whereas the concentrations of all the other analytes were below 100 ng/L. The OPEs in municipal sewage influent and effluent collected at Qinghe waste water treatment plant in the northwest part of Beijing were also determined by the proposed method. All the twelve OPEs except for TPrP, EHDPP and TEHP were detected in both the influent and effluent, and for most OPEs the concentration in effluent was higher than that in influent. TCEP, TCPP, TnBP and TBEP were the major contaminants that occurred at 170-837 ng/L, whereas TCrP, TPHP, TDCP and TEP occurred at 0.274-66.5 ng/L.

**MP012 Spatial and Temporal Trends of MeO-PBDBP Congeners in Herring Gull Eggs from the Laurentian Great Lakes of North America** D. Chen, Carleton Univ, Chemistry, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton Univ, Ottawa, ON, Canada, Dept of Chemistry; R. Letcher, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton Univ, Ottawa, ON, Canada, Carleton Univ, Chemistry; L. Gauthier, S. Chu, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton Univ, Ottawa, ON, Canada; R. McCrindle, Univ of Guelph; D. Potter, Wellington Laboratories. An increasing number of brominated flame retardants and other brominated substances are being reported in herring gull eggs from the Laurentian Great Lakes basin. Yet, in extracts from gulls' eggs, numerous bromide anion response peaks in electron capture negative ion (ECNI) mass chromatograms remain unidentified. Using archived herring gull egg homogenates, we characterized the three major and three minor brominated substances as the congeners of novel methoxylated polybrominated diphenylbenzene (MeO-PBDBP), where four congeners contain five bromines and the other

two contain four and six bromines, respectively. Optimized, semi-quantitative analysis revealed sum concentrations of the MeO-PBDBP congeners ( $\Sigma\text{MeO-PBDBPs}$ ) ranged from  $< 0.2 - 39.1$  ng/g wet weight in pooled egg homogenates (collected in 2009) from fourteen herring gull colony sites across the Great Lakes, with the highest concentration being for Channel-Shelter Island in Saginaw Bay (Lake Huron). Temporal analyses for the time period of 1982 - 2009 indicated that the  $\Sigma\text{MeO-PBDBPs}$  concentrations peaked around early 2000's and then slightly decreased over time in the Channel-Shelter Island egg pool homogenates. To our knowledge, there are no published reports on the environmental presence and sources of MeO-PBDBPs. We hypothesize that these MeO-PBDBPs are degradation products of the polybrominated diphenylbenzenes, e.g., tetradeabromodiphenylbenzene (currently marketed as SAYTEX 120) or polybromo 3P2E. The discovery of MeO-PBDBPs in Great Lakes herring gull eggs indicates their substantial bioaccumulation potential, and raises concerns about their origin, environmental behavior and influences on wildlife and environmental health.

**MP013 Alternative Flame Retardants in the Atmosphere Near the Great Lakes** M. Venier, Indiana Univ, School of Public and Environmental Affairs, Indiana Univ; Y. Ma, Indiana Univ, School of Public and Environmental Affairs; A. Salamova, Indiana Univ, School of Public and Environmental Affairs, Indiana Univ, SPEA; R.A. Hites, Indiana Univ, School of Public & Environmental Affairs. New flame retardants are continuously being introduced into the market to replace commercial products that are either banned or retired. Examples of this trend are 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and *bis*(2-ethyl-1-hexyl)tetrabromophthalate (TBPH), which are replacements for the pentabromo-diphenyl ether commercial mixtures, and decabromodiphenylethane (DBDPE), which is marketed as an alternative for decabromo-diphenyl ether, even though the latter is still largely unregulated. Air (vapor and particulate) and precipitation samples were collected at five sites located on the shores of the Laurentian Great Lakes. The sites ranged from urban (Chicago, Illinois, and Cleveland, Ohio) to rural (Sturgeon Point, New York) and remote (Sleeping Bear Dunes and Eagle Harbor, Michigan). The analytes were an extensive list of alternative flame retardants including hexabromobenzene (HBB), pentabromotoluene (PBT), TBB, and TBPH. Among the brominated benzenes, HBB was detected at the highest levels in the vapor samples with an average concentration of  $3.7 \pm 0.8$  pg/m<sup>3</sup> (average  $\pm$  standard error). The concentrations of this brominated compound were significantly higher than that observed for BDE-47 ( $p < 0.001$ ), which is the main component of the Penta-BDE retired commercial mixture. Unlike the PBDEs, the concentration of several other alternative flame retardants did not track human population density (notably pentabromoethylbenzene was particularly elevated at a remote site near Lake Superior), suggesting the presence of localized sources. TBB and TBPH were detected in all filter samples analyzed at levels similar to those measured for PBDEs. No temporal trends were detected for these two compounds in the 3-year data series available at the moment.

**MP014 Biotransformation of Emerging Flame Retardants: A Case Study of Ring-billed Gulls Breeding in a Pollution Hotspot in the St. Lawrence River, Canada** B. Chabot-Giguere, Environmental Toxicology Research Center (TOXEN), Dept of Biological Sciences, Univ of Quebec in Montreal; R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environment, Canadian Wildlife Service; J. Verreault, Environmental Toxicology Research Centre (TOXEN), Dept of Biological Sciences, Univ of Quebec in Montreal. As a result of the most recent regulations on the penta-, octa- and deca-bromodiphenyl ether (PBDE) commercial mixtures, the use of new, alternative halogenated flame retardant (FR) additives have been on the rise. These alternative or replacement products include 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and decabromodiphenyl ethane (DBDPE), as well as some chlorinated FRs such as Dechlorane Plus (DP, syn- and anti-isomers). These emerging FRs are as yet unregulated in North America and their usage is assumed to increase in the years to come. These new FRs are receiving growing attention as they have recently been detected in wildlife samples, including eggs of fish-eating birds (gulls) from the North American Great Lakes. There are at present important knowledge gaps on the organism fate (e.g., biotransformation) and biological impacts of current-use FRs in wildlife species. This study aims



at understanding the mechanisms of in vitro (liver microsomal) biotransformation of selected PBDEs (BDE-15, -153 and -209) and emerging FRs (BTBPE, DBDPE, syn- and anti-DP) in the ring-billed gull breeding in a known pollution hotspot in the St-Lawrence River basin (Québec, Canada). The ring-billed gull represents an ideal model to study biotransformation of FRs because of its opportunistic feeding habits and hence exposure to a variety of organohalogen pollutants such as the PBDEs and current-use, non-PBDE FRs. Preliminary analyses of ring-billed gull plasma (n=3) have demonstrated the occurrence of major lower- and higher-brominated BDE congeners as well as emerging FR compounds. Highest mean plasma concentration determined for the PBDEs was 7.43 ng/g ww (BDE-47), while that of other FRs was 0.33 ng/g ww (anti-DP). The PBDE/FR analyses in remaining plasma and liver samples are in progress. The catalytic activity determination of cytochrome P450 (CYP) 1A1-like enzymes based on EROD showed a great variability in CYP induction in this breeding colony. For the 23 investigated adult breeding gulls, there was a 3-fold difference between the lowest and highest EROD activities. In vitro biotransformation assays using liver microsomes of these individuals have been used to assess PBDE and non-PBDE FRs degradation. In summary, the results of this research will be of high interest as they will improve our knowledge on the mechanisms of biotransformation of emerging FRs of potential environmental concern in exposed birds from polluted aquatic environments.

**MP015 Emerging Contaminants in Colonial Aquatic Birds of the St. Lawrence River** L. Champoux, Environment Canada, Science and Technology Branch; R.A. Lavoie, Queen's Univ, Biology; J. Rail, Environment Canada, Canadian Wildlife Service; P. Martin, Environment Canada, Science and Technology Branch; R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environme, Canadian Wildlife Service. Since 2004, eggs of Great Blue Herons, Northern Gannets, Herring Gulls, Double-crested Cormorants and Atlantic Puffins have been collected for contaminant analysis as part of on-going monitoring programs over a large stretch of the St. Lawrence River in Quebec, Canada. Eggs of other species (Black Guillemot, Black-legged Kittiwake, Common Tern, Common Eider, Great black-backed Gull, and Razorbill) have also been collected as part of specific research projects. For analysis of emerging contaminants, PBDE and non-PBDE brominated flame retardant (BFR) analyses were conducted at the National Wildlife Research Centre, in Ottawa, by GC/MSD. Perfluorinated compounds were also measured in a smaller number of species. Mean sum BDE/BFRs ranged from 17 ng/g (wet weight) in common terns from a remote site at Iles-de-la-Madeleine in the Gulf of St. Lawrence to 850 ng/g w.w. in herring gulls from the highly urban Montreal area. BFR levels did not increase with trophic level when using all the data but did when freshwater data from the Great Blue Heron were removed. Contaminant levels are higher in the freshwater, more urbanized section of the River, while trophic levels are higher in the estuarine-marine section, possibly because of a more complex food chain.

**MP016 Emerging Halogenated Flame Retardants in Ring-billed Gulls from the St. Lawrence River: Associations Between Ecological Tracers and Diet** E. Caron-Beaudoin, Environmental Toxicology Research Center (TOXEN), Dept of Biological Sciences, Univ of Quebec in Montreal; J. Helie, Geochemistry and Geodynamics Research Center (GEOTOP-UQAM); M. Gentes, Environmental Toxicology Research Centre (TOXEN), Dept of Biological Sciences, Univ of Quebec in Montreal; J. Giroux, Groupe de recherche en écologie comportementale et animale (GRECA), Dept of Biological Sciences, Univ of Quebec in Montreal; J. Verreault, Environmental Toxicology Research Centre (TOXEN), Dept of Biological Sciences, Univ of Quebec in Montreal; R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environme, Canadian Wildlife Service. Some emerging flame retardants (FRs) additives ([e.g., decabromodiphenyl ethane (DBDPE), BTBPE (1,2-bis(2,4,6-tribromophenoxy)ethane), pentabromoethylbenzene (PBEB), etc.], which are replacement products of the recently banned penta- and octa-bromodiphenylether (BDE) mixtures, have been reported to bioaccumulate in wildlife species. Concentrations of PBDEs and some emerging FRs have been reported in herring gull eggs (*Larus argentatus*) from the Laurentian Great Lakes, and were shown to have increased temporally over the last decade. In

order to understand the environmental fate and bioaccumulation dynamics of FRs in aquatic birds, a better understanding of foraging behaviour and space-use is needed. We use the ring-billed gull (*Larus delawarensis*), an omnivorous and opportunistic species breeding close to pollution hotspots in the St-Lawrence River (Montreal, Canada), to investigate the link between diet, space-use and contaminant profiles (PBDE/emerging FRs) of individual gulls using ecological tracers and geolocation data. Feeding ecology and space-use of gulls nesting in a large colony (48,000 pairs) is investigated using bird-borne GPS data loggers that allow detailed tracking (3-5 m) of foraging movements for a 48-72h period. This geolocation technique is used in combination with ecological tracers (stable isotopes of carbon [ $\delta^{13}\text{C}$ ] and nitrogen [ $\delta^{15}\text{N}$ ]) in blood and liver to explain the transfer of nutrients and contaminants within the food web. The  $\delta^{13}\text{C}$  is used to determine the carbon source diversity, while  $\delta^{15}\text{N}$  reveals the trophic position. Results available thus far have shown a wide variability in the stable isotope compositions (C and N) among individuals breeding within this colony, and between males and females. These results suggested high intraspecific variation in dietary preferences. No difference is observed between the  $\delta^{13}\text{C}$  in liver and plasma. However, the  $\delta^{15}\text{N}$  signature is different between those tissues.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are not different between the first and second captures (i.e., after 48-72h) in plasma, which suggested that this time window was shorter than the turnover rate of C and N. Contaminant analyses in plasma and liver are in progress, and results will be presented at the conference. In conclusion, a better understanding of diet documented via the use of stable isotopes and geolocation may be promising for the evaluation of individual contamination profiles in aquatic birds.

**MP017 Leaching of Halogen Free and Brominated Flame Retardants from Polymers** S. Brandsma, J.d. Boer, VU Univ, Institute for Environmental Studies; P. Krystek, MiPlaza, Philips Research Europe and VU Univ, Institute for Environmental Studies; P. Clarke, P. Patel, P. Cusack, ITRI; P. Leonards, Institute for Environmental Studies, VU Univ, Chemistry and Biology, VU Univ, Institute for Environmental Studies. Information about the production, distribution and consumption of flame retardants in electric and electronic (E&E) equipment (plastics) is well described. However, there is a knowledge gap in the amount of flame retardants leaching from plastics of electric and electronic (E&E) equipment to the environment. In the EU funded project ENFIRO, halogen free flame retardants (HFFR) are studied that are viable alternatives to specific commercial brominated flame retardants (BFR). Leaching studies of BFRs from different types of plastics have been described in literature; however, limited information on leaching of HFFRs is available. ENFIRO studies 15 HFFRs of which 6 are metal-based. Metal-based flame retardants are stable in plastic (polymer) products, but can leach, dissociate and enter the environment. Monitoring of the fate of metal-based HFFRs in the environment is difficult as metals can have various sources of emission. Leaching tests of HFFRs from plastics is an alternative method that may contribute to the exposure and risks assessment and understanding of the fate of HFFRs in the environment. The current study shows leaching properties of different HFFRs from polymers in comparison with BFRs. Thereby, the influences of pellets vs. moulded plates and pH on the leaching properties are studied. Two types of leaching protocols were tested. The TLCP protocol, from the USEPA, use worse-case leaching conditions (low pH) to simulate a municipal waste landfill, and studies if waste has toxic characteristics and is hazardous. The second protocol (DIN 38414-S4) determines leaching by water (neutral pH), and has been widely used for regulatory purposes in Europe. We show that no differences in leaching properties between the DIN and TLCP methods for two metal-based PBT pellets were found. However, higher leaching rate coefficients of HFFRs from PBT pellets than PBT moulded plates were found, which is probably a results of the differences in surface:volume ratio and the porosity of the materials. Additional experiments were performed to study the influence of leaching conditions (salinity, humic acids) to simulate different environmental conditions.

**MP018 Occurrence, Distribution, and Transport of (Alternative) Brominated Flame Retardants and Dechlorane Plus in the Global Abiotic Marine Environment** A. Moller, Helmholtz-Zentrum Geesthacht, Institute of Coastal Research, Dept for Environmental Chemistry; Z. Xie, Helmholtz-Zentrum Geesthacht, Helmholtz-Zentrum Geesthacht, Dept for Environmental Chemistry, Helmholtz-Zentrum Geesthacht, Institute of Coastal Research, Dept for Environmental Chemistry, GKSS Research Centre, Research Scientist; R. Sturm, R. Ebinghaus, Helmholtz-Zentrum



Geesthacht, Institute of Coastal Research, Dept for Environmental Chemistry. Organic, mainly halogenated flame retardants (HFRs) have been applied for several decades in various industrial and consumer products to reduce their inflammability and protect humans against fire accidents. Besides their positive effects on fire prevention, they are known to be harmful for the environment. One of the most focused HFRs, the polybrominated diphenyl ethers (PBDEs), are persistent, bioaccumulative, toxic and can undergo long-range atmospheric transport (LRAT). They underwent worldwide banishment since the early 2000s, but there are dozens of other, non-regulated non-PBDE HFRs which haven't received much scientific and public interest even if they have been partly used for several decades, too, or if their production is expected to increase. There is little known on their production and emissions, but they are obviously produced and emitted into the environment worldwide. In the present study, we investigated PBDEs, several non-PBDE brominated flame retardants (BFRs) and the highly chlorinated flame retardant Dechlorane Plus (DP) in air and seawater in the Atlantic and Pacific Ocean, and in the Arctic and the Southern Ocean. High volume air and seawater samples were taken aboard the research vessels Polarstern (Germany) and Snow Dragon (China), Soxhlet extracted and finally measured by GC-ECN-MS. The results showed that HFRs are worldwide distributed in the marine environment, non-PBDE BFRs as well as DP can undergo LRAT into remote regions such as the Arctic and Antarctica, and that the alternative BFRs and DP are meanwhile the predominant HFRs rather than the traditional PBDEs such as BDE-47 and BDE-77. Concentrations were generally highest near the source regions such as Western Europe and East Asia with concentrations in the one- to two-digit pg/m<sup>3</sup> range in air samples (pg L<sup>-1</sup> for water), while Asian concentrations were generally higher than European. Among the non-PBDE BFRs, hexabromobenzene (HBB) and 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) predominated, while especially in the Asian marine environment a wide range of alternative BFRs was observed. DP was detected worldwide, too, while a different transport behaviour of the two stereoisomers – synDP and antiDP – was observed. This study gives important knowledge for the occurrence and transport behaviour including air-seawater exchange of HFRs, especially of non-PBDE HFRs, showing future directions in the research field of HFRs.

**MP019 Capillary Ion Chromatography Mass Spectrometry for Trace Environmental Analysis**

J. Wang, Thermo Fisher Scientific; M. Miller, Thermo Fisher; W. Schnute, Thermo Fisher Scientific. Ion chromatography (IC) has been used extensively as a complimentary separation technique to HPLC. Recently introduced capillary IC (CIC) further improves trace level environmental analysis in matrices when coupled to mass spectrometry (MS). This study involves the use of CIC for the direct analysis of highly polar analytes of environmental concern without labor intensive derivatization or MS incompatible ion pairing; and the modifications of currently available MS electrospray (ESI) source and investigation of interface parameters to improve ionization and transmission efficiency at micro flow levels. This CIC-MS (MS/MS) configuration has been applied for the analysis of endothall (a widely used herbicide) in matrices, ultra-trace perchlorate (low ppt) in rainwater and small organic acids with the details of results to be presented. Significant improvements were observed over previously reported results with respect to method performance such as throughput, sensitivity, precision and system stability. Recommendations for CIC-MS instrumentation and method development will also be presented.

**MP020 Comparative Assessment of GC and GC x GC Characterization Techniques for Estimating Aquatic Toxicity of Complex Petroleum Mixtures**

M.J. Benotti, Battelle, Analytical and Environmental Chemistry; G. Durell, Battelle, Program Manager/Senior Research Scientist; J.R. Thorn, Battelle, Applied Research and Laboratory Operations; A. Frank, E. Strozier, Battelle, Sample Analysis; B. Metzger, Battelle; N. Richardson, Battelle, Environmental Monitoring & Assessment. Evaluation of aqueous toxicity of complex mixtures using chemical characterization, rather than direct toxicity measurements, is challenging given the analytical limitations to chemical identification. This difficulty has been particularly notable when modeling toxicity associated with petroleum mixtures, where chemical analysis has traditionally been conducted using gas chromatography-mass spectrometry (GC-MS) and GC-flame ionization detection (GC-FID), both of which are capable of identifying only a fraction of the compounds typically present. Recently, comprehensive two dimensional GC, combined with either FID (GCxGC FID) or Time of Flight MS (GCxGC-ToF-MS) detectors, has

been shown to greatly expand the resolution of compounds in petroleum products. The greater resolving power of GCxGC techniques is useful for determining sample compositional similarity for attributional studies and may also be valuable for assessing potential aquatic toxicity. Approaches for predicting the aqueous toxicity of complex petroleum mixtures to aquatic organisms range from comparison of Total Petroleum Hydrocarbon (TPH) estimates and empirically-derived toxicity thresholds to models such as PETROTOX, which calculate the dissolution of individual compounds representing various hydrocarbon classes ("blocks") into the aqueous phase and utilizes a Target Lipid Model (TLM) to estimate internal exposures. An aquatic toxicological database is then used to derive toxicological endpoints based on an assumed narcosis mode of action and general additivity. This study evaluates the relative performance of four analytical techniques (GC-FID, GC-MS, GCxGC-FID and GCxGC-ToF-MS) by comparing the predicted aquatic toxicity estimates derived from different toxicity assessment approaches across a range of crude and refined petroleum mixtures. A number of petroleum products including various crude oils (including North Slope crude, Campeche Sound heavy/light crude, Kuwaiti Mardomah crude) and refined products (including diesel fuel, kerosene, and gasoline) were included in this study. Predicted toxicity results were compiled for each of the evaluated analytical approaches, and toxicity thresholds derived for each sample. Using the GCxGC-ToF-MS results to benchmark the predicted toxicity estimates of the other analytical techniques along with cost data, a comparative assessment of the various analytical techniques will be presented.

**MP021 Mass Balance Analysis on AFFFs: TOF-CIC and LC-MS/MS**

L.W. Yeung, B. Weiner, S.A. Mabury, Univ of Toronto, Dept of Chemistry. Aqueous film forming foams (AFFFs) were developed in the 1960s by the United States Navy and the 3M company in order to extinguish fires involving inflammable liquid fuels, such as kerosene and gasoline. They have been widely used for emergency or training purposes at military bases, fire Depts, and airports. Fluorinated surfactants are present in AFFFs at 1-5% by weight, which were in the past dominated by perfluorooctanesulfonate (PFOS). However, due to concerns of toxicity and bioaccumulation, PFOS was discontinued in 2002 in North America by 3M. Fluorotelomer-based surfactants that include the 6:2 fluorotelomer sulfonate (6:2 FTS) backbone have since then replaced PFOS as the fluorinated surfactants used in AFFFs. These fluorinated surfactants are considered to be biodegradable and it has been suggested they are not bioaccumulative. Due to proprietary reasons, little is known about the chemical composition of currently available AFFFs. Recent developments in liquid chromatography and mass spectrometry allow the identification and quantification of trace levels (sub parts-per-billion) of perfluorinated components in a sample. Up to date, over 50 poly- and per- fluorinated carboxylates/sulfonates/phosphates have been identified in environmental samples. However, quantitative measurements of the perfluorinated content can only be performed when components are known and all authentic standards are available. In this study, a combined combustion ion chromatography, namely Total Organo Fluorine – Combustion Ion Chromatography (TOF-CIC) is used to measure the total organic and inorganic fluorine content in AFFF samples. The foams were further analyzed for 22 known poly-/per- fluorinated carboxylates/sulfonates using HPLC-MS/MS in electrospray ionization (negative mode). Preliminary results suggest that inorganic fluorine was present at

**MP022 Development of an Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry Method for Quantifying Fatty Acids Without Derivatization**

G. Wei, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; E.Y. Zeng, Guangzhou Institute of Geochemistry, State Key Lab of Organic Geochem. A simple, rapid and sensitive method based on ultra-high performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) without prior derivatization was developed for quantification of 41 components of fatty acids (FAs, from C10 to C30, consisted of saturated, branched, mono- and polyunsaturated components including trans-cis isomers and double-bond positional isomers). Instrumental analysis was performed in the negative electrospray ionization mode with pseudo-multiple reaction monitoring transitions by selecting [M-H]<sup>-</sup> as the precursor ions as well as the product ions. Through internal standard calibration, this method obtained satisfactory recoveries for the target FAs (65–119%) and the surrogate standards C18:1-d2 and C16:0-d30 (76–109% and 79–106%, respectively) from spiked blank samples, with relative standard deviations all less than 20%. The recoveries

of the surrogate standards C18:1-d2 and C16:0-d30 in sediment and soil samples were in the ranges of 66–86% and 55–89%, respectively. Limits of instrumental quantification (defined as the quantity with a signal-to-noise ratio of 10 in a standard solution) varied between 2 and 50 pg on column, demonstrating satisfactory detection sensitivity. Compared with conventional gas chromatography/mass spectrometry (GC/MS) techniques requiring complicated derivatization of FAs into volatile derivatives, the UHPLC/MS/MS method developed in the present study required simpler sample processing steps, resulting in good recoveries for a variety of FAs (C10:0–C30:0). The UHPLC/MS/MS method also exhibited sufficient detection sensitivity and superior reproducibility, and had advantage in shorter analysis time over GC-MS for all target FAs. Therefore, UHPLC/MS/MS may serve as a useful tool for quantifying FAs in various fields.

**MP023 Toxicological Applications of Cryogenic Laser Ablation Inductively Coupled Plasma Time of Flight Mass Spectrometry (CLA-ICP-TOF-MS)** S.M. Lev, M. Monk, Towson Univ, Urban Environmental Biogeochemistry Laboratory. The analysis of acid digested bulk tissue samples by ICP-MS is a primary method for toxicologists when trying to characterize the uptake and storage of a trace metal by an organism. This approach is sensitive, reliable and reproducible but requires the homogenization of the tissue for analysis. This procedure does not provide any information on the distribution of the analyte of interest within the tissue sample. Sub-sampling is a possible alternative method for preserving distribution data but in many cases sub-sampling on the scale necessary for useful information is not an option due to sample size limitations. By coupling a laser ablation system equipped with a cryogenic cell to an Inductively Coupled Plasma Mass Spectrometer (CLA-ICP-MS), it is possible to achieve high-resolution trace metal distribution data on toxicological tissue samples. A cryo cell is critical for proper ablation of soft tissue samples by LA-ICP-MS. The system used for the work presented is a commercially available cryo cell from GeoMed Analytical capable of temperatures as low as -30°C, coupled to a GBC Scientific ICP-Time of Flight-MS (ICP-TOF-MS). The speed of the TOF system is an ideal platform for the analysis of the transient signal produced by laser ablation and has detection limits that are comparable to traditional ICP-MS (i.e., low ppb range). This system is suitable for the ablation of a range of tissues with a spatial resolution of 10 microns for most elements. Results will be presented to demonstrate the detection limits and spatial resolution of CLA-ICP-TOF-MS for a variety of elements in a range of tissue types. Recommended conditions for ablation, suggestions for drift correction, and mass bias correction will also be presented.

**MP024 Two-dimensional Liquid Chromatography – High Resolution Mass Spectrometry for the Analysis of Polar Organic Contaminants in the Aquatic Environment** G.J. Getzinger, Duke Univ, Nicholas School of the Environment; L. Ferguson, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Nicholas School of the Environment, Pratt School of Engineering, Dept of Civil & Environmental Engineering. The emergence of high-resolution mass spectrometry (HRMS) as an analytical tool in environmental chemistry has expanded the scope of research into fate and transport of organic contaminants in complex mixtures. Recent advances in instrumentation have allowed for new approaches in trace organic analysis, including non-target contaminant screening, transformation product identification, retrospective sample analysis, and increased certainty in the identification and quantitation of legacy and emerging contaminants. Currently, most environmental applications of HRMS use the unique capabilities of these instrument platforms (e.g., high mass accuracy and exceptional tolerance for sample complexity) to monitor ultra trace (ppq-ppt) levels of polar and semi-polar organic pollutants by incorporating high performance liquid chromatography (HPLC) and electrospray ionization (ESI). However, HPLC-ESI-MS analysis of complex environmental samples such as wastewater often suffers from matrix-related signal suppression and poor chromatographic resolution of target analytes from background contaminants. To address these limitations while maximizing the performance of the LTQ-Orbitrap HRMS/MS instrumentation, we have developed a comprehensive two-dimensional liquid chromatography (2D-LC) method for the HRMS analyses of emerging contaminants in highly complex environmental samples. This method was optimized and implemented for the analysis of both target (e.g. xenoestrogens, surfactants, pesticides, herbicides, and fungicides) and non-target anthropogenic contaminants in wastewater and ground water samples from Kiawah Island, SC. In these analyses, the capabilities of 2D-LC-HRMS in environmental analysis, were compared

to traditional sample enrichment and clean-up strategies upstream of single dimension LC-HRMS. To reduce the influence of matrix interferences and achieve the necessary sensitivity and specificity demanded in environmental analysis, contemporary LC-MS methods often utilize targeted sample enrichment and clean-up. However, these methods often result in significant losses of non-target and unknown sample components. Our results indicate that the chromatographic resolution achieved by 2D-LC coupled with the high performance capabilities of the LTQ Orbitrap Velos ( $R > 100,000$ , mass error  $< 2$  ppm) provides a new, unique, and powerful tool for the monitoring of target and non-target analytes in highly complex samples.

**MP026 Mercury Emissions from Soils as Affected by Soil Moisture and Sterilization** R. Pannu, Univ of Saskatchewan, Soil Science, Acadia Univ, KCI Center; N. O'Driscoll, Acadia Univ, Earth and Environmental Sciences; S.D. Siciliano, Univ of Saskatchewan, Dept of Soil Science; J. Dalziel, Environment Canada, Air Quality Sciences Section. Mercury is a globally dispersed pollutant due to its long (1.5 – 2 years) atmospheric residence time (Munthe et al., 2001; Lindberg et al., 2007). Accurate prediction of global and regional mercury flux is important for mitigating the mercury burden of ecosystems. Natural emissions and re-emissions of Hg(0) from soils have been identified as a major contributor to the global mercury budget and conservative estimates of global mercury fluxes suggest a total of 700 to 1000  $\text{ta}^{-1}$  volatilized from soils (Lindqvist, 1991). There are large variations in published mercury flux values from soil surfaces due to number of uncertainties associated with flux measurements as affected by soil moisture. Rising soil water content can promote the aqueous reduction of Hg(II) to Hg(0) with subsequent volatilization to atmosphere. This research quantifies the effect of soil moisture (measured as percent water filled pore space (WFPS) – 15, 30, 45 and 60%) on the kinetics of elemental mercury Hg(0) production and volatilization to the atmosphere in 13 soils obtained from Nova Scotia, Canada under controlled conditions. The soils maintained at 45% WFPS were found to have the highest Hg(0) production while negligible mercury volatilization was found at near saturation levels (60% WFPS). First-order kinetic model using Maquardt iteration method fitted the cumulative mercury produced data well ( $R^2 = 0.90$  to  $0.99$ ,  $P < 0.001$ ) in all the studied soils. Cumulative mercury production curves in soils followed a distinct exponential pattern initially at all PWFPS with mercury leveling off after 5-8 hours. Multiple linear regression analysis with reaction rate constants (k) as dependent variable and soil properties (total Hg, organic carbon, pH and electrical conductivity) as independent variables indicated that rate of reaction can be predicted from a linear combination of soil properties at 15 ( $R^2 = 0.308$ ,  $P > 0.05$ ), 30 ( $R^2 = 0.265$ ,  $P > 0.05$ ), 45 ( $R^2 = 0.288$ ,  $P > 0.05$ ) and 60% WFPS ( $R^2 = 0.306$ ,  $P > 0.05$ ). We will also present the data showing effect of soil irradiation on Hg(0) production as influenced by soil biotic activity.

**MP027 The Effect of Tidal Flushing on Mercury Fate and Biogeochemistry in Intertidal Sediments** J. Canario, IPIMAR, Aquatic Environment Dept, INRB IP/IPIMAR, Aquatic Environment and Biodiversity; M. Caetano, INRB IP/IPIMAR; L. Poissant, Environment Canada; C. Vale, INRB IP/IPIMAR; N. O'Driscoll, Acadia Univ, Acadia Univ, Dept of Earth & Environmental Sciences. Intertidal environments are characterized by complex subsurface hydrologies regulated by topography, sedimentology, groundwater discharge, as well as tidal pressure and wave action. In these environments vertical flow at ebb tide increase hydrostatic pressure at depth and forces the release of pore water to the surface. In order to assess how this force influences mercury fate in intertidal sediments and the associated biogeochemical processes, several inundation experiments were performed in vegetated and non-vegetated intertidal sediments in three sites of the Tagus Estuary (Portugal). Sediments and overlying water were collected at a high temporal resolution after inundation at each sampling site. Dissolved gaseous mercury (DGM) and water-to-air volatilization was measured using a floating dynamic flux chamber. In the laboratory, water (pore water – PW – and overlying water – D) samples were analysed for dissolved mercury species ( $\text{Hg}_D$  and  $\text{Hg}_{PW}$ ), methyl mercury (MeHg), dissolved organic carbon and suspended particulate matter (SPM) content. Salinity, dissolved oxygen and pH were measured in situ. Solid sediments were analysed for Hg, MeHg and organic carbon content. In vegetated sediments Hg and MeHg content were also determined in the root biomass. Concentrations of dissolved mercury species in flooded water increased up to 10 times during the first 5 minutes of tidal flooding in vegetated and non-vegetated sediments. Concurrently MeHg concentrations decreased in solids and in pore

waters. A strong correlation ( $R = 0.99$ ;  $p < 0.01$ ) was found between mercury volatilization from water and DGM concentrations indicating the escape of recently formed  $Hg^0$  to the atmosphere. During the inundation of vegetated sediments MeHg concentrations in roots decreased as much as 31% in the first 8 minutes after the inundation. This suggests that MeHg is far mobile that would be expected if the sequestration processes in roots was dominated by affinity to cysteine-rich peptides. The short-time variations registered in this work indicates a shift in the mercury equilibrium between pore waters, solid sediments and root surfaces. As water floods salt marsh sediments, MeHg, escapes from the sediments being potentially available to biota living those environments. The results obtained in this study clearly points to the importance of tides in Hg and MeHg export from sediments, DGM formation and Hg volatilization in shallow meso- and macrotidal estuaries.

**MP028 Seasonal Variation of Methylmercury Sediment/Water Fluxes in a Meso-tidal Estuary (Tagus-SW Europe)** R. Cesario, INRB-IPIMAR National Institute of Biological Resources, Dept of Environmental and Biodiversity; M. Caetano, INRB IP/IPIMAR; J. Canario, IPIMAR, Aquatic Environment Dept, INRB IP/IPIMAR, Aquatic Environment and Biodiversity; C. Monteiro, M. Nogueira, INRB-IPIMAR National Institute of Biological Resources; C. Vale, INRB IP/IPIMAR. Methylmercury (MeHg) is the most concern mercury species to aquatic systems due to its ability to bioaccumulate in food webs. Methylmercury is mainly produced in sediments by microorganisms and then can be exported to water column by molecular diffusion or other transport processes. The Tagus estuary in Portugal is one of the most Hg contaminated in SW Europe. Recent works point to the existence of 23 ton of Hg stored only in the first 5-cm sediment depth. In a recent work with 92 surface sediments it was proved that MeHg concentrations varied seasonally with more 32% of MeHg found in summer season. In spite of this findings very little is known about MeHg variation in deeper sediments and mainly if there is any seasonally variation of MeHg sediment/water fluxes. This issue is particularly important because an increase of these fluxes can lead to an increase of aquatic life exposure to MeHg in warmer season. In order to clarify these subjects sediment cores and overlying water were collected in Summer 2010 and Winter 2010/2011 at four sites from Tagus estuary with different degree of mercury contamination. Pore waters were separated from solids by centrifugation and all water and solid samples were analyzed for total mercury, MeHg and other interpretative parameters. Results obtained in both solids and pore waters confirm previous findings found in surface sediments, i.e., higher proportion of MeHg in summer months, not related to the degree of Hg contamination. The amount of MeHg in the all estuary increased up to 20% that may affect sediment/water fluxes. Noteworthy, an upward MeHg diffusive flux was observed in winter that was inverted in summer. These results indicates that, although MeHg production increased in summer, overlying water MeHg levels increase in higher proportion compared to the levels in pore waters. A decrease in demethylation in the water column during summer may explain why levels of MeHg were higher. It is well documented that the environmental conditions found in Tagus waters in summer (decrease of redox potential, lower dissolved oxygen, etc) disfavors demethylation reactions.

**MP029 Merganser: An Empirical Model Linking Atmospheric Deposition and Watershed Features to Mercury Levels in Fish and Loons in New England** A.C. Simcox, US Environmental Protection Agency, Region 1; J. Shanley, R. Moore, R. Smith, US Geological Survey; E.K. Miller, Ecosystems Research Group, Ltd; N. Kamman, Vermont Dept of Environmental Conservation; D. Nacci, US Environmental Protection Agency; K. Robinson, US Geological Survey; J. Weiss, J.M. Johnston, M. Hughes, US Environmental Protection Agency; C. Johnston, US Geological Survey; D. Evers, K. Williams, Biodiversity Research Institute; J. Graham, Northeast States for Coordinated Air Use Management; S. King, New England Interstate Water Pollution Control Commission. EPA and a team of mercury researchers in the northeast US recently completed development of a GIS-based model called MERGANSER (Mercury Geospatial Assessments for the New England Region). The model, which was developed with data gathered over a decade, uses an empirical approach to relate atmospheric Hg deposition and lake and watershed characteristics to Hg concentrations in fish and fish-eating wildlife (common loons). The model identifies statistically significant watershed features, such as total Hg deposition, watershed area, percent forest canopy and wetland area, that, especially in combination, heighten the risk of high mercury levels in biota. The model estimates the

probability that a specified fish species and/or common loon in a given New England lake will exceed a threshold Hg concentration and gives an estimate of Hg concentration in fish tissue and loon blood with error bounds. These qualitative and quantitative measures can be used to identify areas throughout New England where human-health impacts (via fish consumption) and ecological impacts may be occurring. MERGANSER includes 4,404 New England lakes of 8 hectares (about 20 acres) or more in size. A total of 56 independent spatial variables were tested for statistical significance as predictors of Hg concentrations in fish tissue and loon blood. Of these candidate variables, nine were identified as significant predictors. MERGANSER also includes three interaction terms where the effect of one independent variable on the dependent variable (i.e., fish Hg or loon Hg) depends on the value of another independent variable. All of the interaction terms included total Hg deposition. The model explains about 63 percent of the variance in Hg levels of all fish and loon samples included in the model, which is considered a good result given the complexity of Hg transport and fate in the environment. MERGANSER can also be used to explore changes in Hg levels in fish and loons resulting from changes in atmospheric Hg deposition, temperature changes due to climate change, or changes in land use, and to identify optimal locations for monitoring. Additionally, MERGANSER provides a modeling approach for other regions of the US.

**MP030 Trends in Mercury Deposition Downwind of Major Canadian Point Sources** J.L. Kirk, Environment Canada; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, National Water Research Institute; M. Evans, Environment Canada; H. Hintelmann, Trent Univ; F. Yang, X. Wang, A. Gleason, Environment Canada; J. Cory, Univ of Waterloo; G. Lawson, C. Tunks, Environment Canada. Increased rates of atmospheric inorganic mercury (Hg) deposition have been shown to increase methyl mercury (MeHg) concentrations in fish; however the relative importance of regional versus local Hg sources are generally not known. In Canada, estimates from the global/regional atmospheric heavy metals model (GRAHM) indicate that while in most regions only ~2% of Hg deposition originates within Canada, there are "hotspots" where ~60% of deposition originates from local sources. As part of the Clean Air Regulatory Agenda (CARA) Hg science program, we are quantifying Hg deposition to aquatic ecosystems from local sources using analysis of dated lake sediment cores. In 2009-2010, sediment cores, water samples, and catchment soils were collected from: 18 lakes located varying distances from a metal smelter located at Flin Flon, Manitoba and coal-fired power plants in central Alberta; 5 lakes in Kejimikujik National Park, Nova Scotia where MeHg concentrations in biota are known to be high despite the absence of local point sources; and 5 lakes at the Experimental Lakes Area in Northwestern Ontario, where Hg deposition rates should represent regional background. In Flin Flon lakes, total Hg (THg) fluxes began increasing in the ~1930s, when metal smelting began in this region and peaked in the late 1980s-early 2000s, reaching up to 6529  $\mu\text{g}/\text{m}^2/\text{year}$  in lakes near the smelter. However, THg fluxes dramatically decreased with distance from the smelter and flux ratios ( $\text{FR}$ ;  $\text{HgF}_{\text{post 1990}}/\text{HgF}_{\text{pre 1990}}$ ) were 27-47 in lakes within 5 km of the smelter, 10-17 within 40 km, and only 3-5 70 km away. Lake water THg concentrations also decreased with increasing distance from the smelter ( $r^2=0.51$ ,  $p=0.03$ ), but were low ( $1.08 \pm 0.40$  ng/L) indicating that most of the Hg emitted from the smelter rapidly deposits to lake sediments. In contrast, in Alberta lakes, Hg fluxes showed no trend with distance from the power plants and FRs were only 2-6. Lake water THg concentrations also did not vary with proximity to the power plants and averaged only  $0.46 \pm 0.34$  ng/L. These results suggest that the coal fired power plants are emitting fine particulate-bound or gaseous  $Hg(0)$  species which travel long distances after emission. Catchment soils are currently being analyzed for THg and lithogenic elements so that catchment and atmospheric Hg inputs to each lake can be distinguished. Results from the Kejimikujik and ELA lakes will also be presented.

**MP031 A Comparison of Mercury Concentrations in Sediment, Water, and Biota of Lakes Near Two Major Hg Emitters in Western Canada** K. Bielefeld, Environment Canada, Water Science and Technology Directorate, Univ of Saskatchewan, Biology, Univ of Saskatchewan, National Hydrology Research Center; M. Evans, Environment Canada; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute; J.L. Kirk, Environment Canada, Research Scientist, Environment Canada; J. Keating, Environment Canada, Water Science and Technology Directorate. Environmental contamination due to anthropogenic mercury (Hg) releases is an issue of increasing global



concern. As a part of the Canadian government's commitment to reduce Hg emissions, Environment Canada is investigating the factors affecting spatial and temporal variability in Hg concentrations in sediment, water, and biota of lakes near two major Hg emitters in western Canada. Here we assess the localized impact of Hg pollution from two major industrial emitters in Canada – a base metal smelter at Flin Flon, Manitoba located on the Precambrian shield and a cluster of 4 coal-fired power plants in central Alberta's boreal plain. Shield lakes are relatively isolated, surrounded by boreal forest and have received emissions from the Hudson Bay Mining and Smelting complex in Flin Flon, MB since 1930. Central Alberta lakes are highly productive and receive inputs from the surrounding urban and agricultural landscape in addition to emissions from a complex system of four large coal-fired power plants, in operation since the 1950's. We collected northern pike (*Esox lucius*), forage fish, benthic invertebrates, zooplankton, water and sediment for Hg analysis from lakes at both study locations. Sediment Hg concentrations are very high in lakes in the immediate Flin Flon area but rapidly diminish with distance while sediment Hg levels are low in all of the highly productive Alberta lakes. Despite notable differences in sediment Hg concentrations, lake productivity, and watershed characteristics between each area, northern pike in the immediate Flin Flon area do not reflect high sediment Hg concentrations and, like Alberta pike, are below consumption guidelines of 0.5 µg/g. Furthermore, lakes ~ 70km northwest of the Flin Flon smelter have exceedingly high pike Hg concentrations despite low sediment Hg and distance from source. Also being discussed is the use of stable isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) to characterize food webs and develop biomagnification rates within each lake. These findings will highlight the importance of implementing emissions controls specific to ecological and environmental factors and will have key implications for future industry.

**MP032 Factors Affecting Mercury Biomagnification Through Lake Food Webs in the Canadian High Arctic** G. Lescord, Canadian Rivers Institute, Univ of New Brunswick, Biology Dept; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; D. Muir, J. Kirk, X. Wang, G. Lawson, Environment Canada; N. O'Driscoll, Acadia Univ. Mercury (Hg) concentrations in landlocked Arctic char (*Salvelinus alpinus*) in the Canadian High Arctic can vary up to 4-fold between neighboring populations. Since Arctic lakes receive most of their Hg from wet deposition and summer snowmelt, their abiotic and biotic Hg concentrations may be affected by the area of the catchment and lake. As in lower latitude systems, chemical or biological features such as dissolved organic carbon (DOC), pH, and the feeding ecology of an organism may also affect Hg uptake and transfer through lake food webs. This study is comparing Hg concentrations in the food webs of six lakes on Cornwallis Island, Nunavut, each with differing physical and chemical features. For example, lake areas, DOC, and chlorophyll concentrations range between 0.13-1.3 km<sup>2</sup>, 0.59 -2.08 mg/L, and 0.05-1.10 µg/L, respectively. The lakes were sampled repeatedly for sediments, invertebrates, and char (August only) during and after the annual spring melt in 2010. Water samples were also collected weekly from each lake to assess temporal changes in Hg concentrations. All samples were analyzed for methyl Hg (MeHg) and total Hg (THg) and the food webs were characterized using stable isotopes; nitrogen isotopes are used to assess trophic level and carbon and sulfur isotopes are used to assess energy source. Mean THg concentrations (0.05-1.38 µg/g, wet wt) in char varied significantly between these lakes (Kruskal-Wallis  $p < 0.001$ ). Analysis of stable isotopes in biota and MeHg of the lower-trophic-level biota and abiotic samples are ongoing. Preliminary water data show significant differences between several lakes in MeHg (0.01-0.16 ng/L) and THg (0.28-1.1 ng/L), with higher concentrations and percent MeHg in Meretta Lake, potentially due to local nutrient inputs and its higher productivity. Rates of Hg biomagnification through the food web will be quantified by regressing log MeHg versus nitrogen isotopes and these relationships will be compared against results from other studies. Multivariate analyses will be performed to determine the factors that best predict Hg concentrations in char. Results from this study will improve our understanding of the factors underlying the variability in fish and invertebrate Hg in High Arctic lakes and offer critical baseline information for future studies to assess how these systems are changing in response to climate change.

**MP033 Evaluation of Hydro-climatic Drivers of Contaminant Transfer in Aquatic Food Webs in the Husky Lakes Watershed, Northwest Territories, Canada** N. Gantner, Univ of Victoria, Dept of Geography; T. Prowse, F. Wrona, Water & Climate Impact Research Centre, Environment

Canada; H. Hintelmann, Trent Univ, Environmental and Life Sciences; J. Reist, Freshwater Institute, Fisheries and Oceans Canada. Climate change in the Arctic has the potential to affect mercury concentrations ([Hg]) in freshwater fishes. Mercury can accumulate in apex-predator fish muscle to concentrations exceeding those considered safe for subsistence consumption by humans. Fish species such as Lake trout are typical apex-predators of Arctic lakes and can be a significant source of food for local indigenous peoples. The influence of abiotic factors, which are climate influenced, and biological parameters on Hg accumulation in apex-predators are not well understood. Further, a good understanding of sources of Hg to and processes within water column and food webs is still lacking. Our study aims to investigate the interactions of cryosphere, food webs and Hg transfer in four freshwater systems in the Inuvialuit Settlement region (Canada). The selected Big, Yaya, Noell, and Husky Lakes systems represent a range of cryospheric and ecological characteristics, as well as Hg delivery (marine-, riverine- or freshwater-derived). We investigate how those characteristics affect Hg transfer and fractionation. Biotic and abiotic fractionation of Hg isotopes has recently been shown to occur in aquatic environments and Hg isotope ratios (IRs) are proposed as useful tool to elucidate processes and possibly sources of Hg. All lakes are frequented by the Inuvialuit communities Inuvik and Tuktoyaktuk for subsistence fishing. Sampling includes tissues from harvested fishes, non-target fishes, and food web compartments (periphyton, zooplankton, and benthos). Biological parameters of fishes (age, length, weight, diet) are recorded and invertebrates separated by species. Sample analysis includes total Hg (THg), monomethylHg (MeHg), and stable isotopes of carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and Hg ( $\delta^4\text{Hg}$ ). Hg IRs are analyzed by multicollector inductively coupled plasma mass spectrometry (MC-ICP/MS). Hg mass independent fractionation (MIF;  $\Delta^{199}\text{Hg}$ ) and mass dependent fractionation (MDF;  $^{202}\text{Hg}$ ) are calculated and evaluated against conditions in the water column, food web transfer and the potential difference in Hg delivery. [THg] detected in harvested fishes will be compared to consumption guidelines. Food web transfer of MeHg will be evaluated against cryospheric conditions and related lake productivity. We will discuss our preliminary findings in the context of the currently emerging use of Hg IRs with particular focus on implications for future research efforts in a changing Arctic environment.

**MP034 Influences of Ingestion Rate on Dietary Accumulation of Mercury in Tilapia (*Oreochromis niloticus*) and the Implications on Somatic Growth Dilution** R. Wang, Section of Marine Ecology and Biotechnology, Division of Life Science, The Hong Kong Univ of Science and Technology (HKUST); W. Wang, Hong Kong Univ of Science and Technology. Dietary ingestion constitutes a major pathway for mercury (Hg) accumulation in freshwater fish, and so the ingestion rate (IR) may greatly influence the Hg bioaccumulation through its effect on Hg influx and other biokinetic processes. To explore the complex influence of IR, we conducted long-term bioaccumulation experiments by accurately controlling the IRs in the freshwater tilapia (*Oreochromis niloticus*). The dietary accumulation of both inorganic mercury (Hg(II)) and methylmercury (MeHg) in tilapia under different IRs was monitored over a period of 30 days by feeding the fish with uniformly radiolabeled crustaceans. The biokinetic parameters under various IRs were also concurrently determined. When the IR increased from 0.01 g g<sup>-1</sup> d<sup>-1</sup> to 0.12 g g<sup>-1</sup> d<sup>-1</sup>, the dietary assimilation efficiency of Hg(II) in the tilapia decreased by 43% while the elimination rate increased by a factor of 1.8; both biokinetic changes slowed down the overall Hg(II) bioaccumulation. In contrast to Hg(II), the biokinetics of MeHg was not significantly influenced, but its bioaccumulation increased disproportionately with increasing IR. We then employed a biokinetic model to simulate the long-term mercury bioaccumulation patterns in tilapia at various IRs. The modeling results indicated that the growth effect could not be ignored in long-term accumulation process, that a rapid growth of fish driven by food availability could significantly reduce the MeHg concentrations in the tilapia. Under the tested condition in this study, a 2.6-fold higher IR could result in 1.7-fold reduction of the MeHg (but not Hg(II)) concentrations in fish primarily as a result of the high sensitivity of MeHg bioaccumulation to fish growth. Our results demonstrated for the first time the contrasting influences of fish feeding on the long-term bioaccumulation of Hg(II) and MeHg and that the somatic growth dilution was much more likely to occur for MeHg than for Hg(II).

**MP035 Trophic Transfer Dynamics of Polychlorinated Biphenyls (PCBs) and Cyclic Volatile Methylsiloxanes (cVMS) in Lake Pepin, MN,**

**USA R.M. Seston**, Dow Corning Corporation, Health & Environmental Sciences; D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101); D. McNett, Dow Corning Corporation, Health and Environmental Sciences; J.A. Durham, Dow Corning Corporation, Health & Environmental Sciences; P.W. Bradley, M.J. Zwiernik, Michigan State Univ, Dept of Animal Science. The bioaccumulation potential, or 'B', of a compound is one of the criteria used to classify substances as being PBT (persistent, bioaccumulative, and toxic) materials in the environment. Bioaccumulative substances are defined as substances that biomagnify within a food web. A compound is said to biomagnify when lipid-normalized concentrations of accumulated chemical residues in biological organisms increase with increasing trophic position. Based on this definition, the most relevant criterion for assessing a compound's 'B' potential is the trophic magnification factor (TMF), and the most conclusive evidence to demonstrate that a chemical substance biomagnifies is a TMF > 1. Previous research aimed to determine the 'B' potential of several cyclic volatile methylsiloxanes (cVMS) was conducted in Lake Pepin, Minnesota, USA and concluded that biomagnification of cVMS materials was not occurring. Rather, cVMS materials had TMF values that were < 1.0 (range 0.1 to 0.3), indicating that concentrations of cVMS materials decreased with increasing trophic level and that trophic dilution was occurring across the aquatic food web. The objective of the present study was to verify that Lake Pepin is appropriate for conducting TMF studies by evaluating the trophic magnification behavior of polychlorinated biphenyls (PCBs), which are considered legacy 'B' contaminants and whose environmental behavior has been extensively studied. A subset of moderate to heavily chlorinated PCB congeners were quantified in biota at various trophic levels and TMFs were calculated. As expected, TMFs for the selected PCB congeners were greater than 1, ranging from 1.5 to 5.1. These results suggest that Lake Pepin is appropriate for TMF studies and that the TMFs of < 1.0 for cVMS compounds in the same aquatic food web appear to be valid. Moreover, it has been suggested that TMFs may be improved in instances where there is limited environmental data by using a standardizing benchmark to calibrate trophic level assignments. When PCB 180 data from the present study were used to calibrate trophic level assignments in the aquatic food web of Lake Pepin, the TMFs of the cVMS materials were lower than the originally reported values. Thus based on this data, select PCB congeners behave as expected while cVMS do not appear to be bioaccumulative in Lake Pepin.

**MP036 Measured Vegetation Uptake Factors for Selected Inorganics in Wetlands** R. Devries, AMEC; T. Sorell, AMEC, Life Sciences; J. Robb, D. Cooke, AMEC. Plant uptake studies were conducted as part of a multi-phase ecological risk assessment in wetlands impacted by historic coal ash ponds. Co-located soil and plant samples (boneset, dewberry and birch leaves or cattail rhizomes) were collected and analyzed from locations near the source areas and from an identified Reference Area. Evaluation of mathematical relationships between soils and plant concentrations of selected coal combustion-related inorganics indicated minimal or no plant tissue increases in response to elevated soil concentrations or compared to background. Where plant tissue concentrations did increase in response to higher soil loading (such as for boron and molybdenum in cattail rhizomes and molybdenum in leaves), the increases were small and described by a log-log relationship in which the vegetation uptake factor (VUF) decreased with increasing soil concentrations, a pattern consistent with published models. This relationship reflects greatly reduced VUFs associated with higher soil levels. Thus the use of fixed VUFs in food chain modeling will typically lead to overestimates of the plant tissue concentrations, especially in areas of higher soil impact, and should be avoided. Overall, the generally flat relationship between wetland soil and plants indicates that plant uptake may be an insignificant or incomplete food chain transfer pathway.

**MP037 Relationship Between Water Hardness and Toxicity of Major Anions: Implications for Water Quality Guidelines for Individual Anions and TDS** J. Elphick, Nautilus Environmental; G. Gilon, Independent; B. Chalmers, Mining Association of BC; J. Baker, Nautilus Environmental; K. Bergh, All North Consultants; H. Bailey, Nautilus Environmental. Recent investigations have demonstrated that the toxicity of anionic components of total dissolved solids, including sulphate, chloride and nitrate, exhibit considerably elevated toxicity under soft water conditions compared with higher hardness conditions. This relationship appears to occur either as a result of competition by ions for uptake sites

at biological membranes, or as a result of the effect of ions that contribute to hardness, in particular, calcium and magnesium, on the permeability of these membranes. The presence of major anions at elevated concentrations almost always co-occurs with elevated hardness, since dissolved anions are balanced by concentrations of cations, which include those constituting hardness. Thus, the interaction of water hardness on toxicological effects of anions is highly relevant in the context of evaluating environmental risk of total dissolved solids. This presentation summarizes the relationships that have been established between water hardness and toxicity of anions, and compares the relationships between anions to establish whether they result from similar mechanisms. The findings have relevance in the context of setting water quality guidelines for the individual anions, as well as for total dissolved solids.

**MP038 Sensitivity of Gray Treefrog (*Hyla versicolor*) Embryos, Hatchlings, and Tadpoles to Sulfate and Chloride Exposure** B.K. Williams, E.E. Little, H.J. Puglis, E.L. Beahan, USGS, Columbia Environmental Research Center. Identifying sensitive life stages is a critical step in understanding the toxicity of major ions to aquatic life. This need is especially great for infrequently tested organisms such as amphibians. Although amphibian larvae are commonly found in surface water habitats threatened by elevated sulfate and chloride levels, toxicity data are generally lacking, particularly for sulfate. In addition, there is inconsistent evidence that young larvae are the most sensitive aquatic life stage to major ion toxicity, as is often assumed. We are performing standardized 96-hour acute tests with gray treefrog (*Hyla versicolor*) embryos, hatchlings, and young tadpoles, in order to 1) estimate acute LC50 values for the anions sulfate ( $\text{SO}_4^{2-}$ ) and chloride ( $\text{Cl}^-$ ), and 2) identify any life-stage-associated differences in sensitivity to sulfate and chloride exposure. Results thus far indicate that gray treefrog embryos and hatchlings show comparable sensitivity to each of the tested anions, with LC50 values greater than 3200 mg/L for sulfate and greater than 2000 mg/L for chloride. The results of these multi-stage tests will inform the selection of appropriate life stages for future testing efforts, and will help ensure that resultant water quality guidelines are protective of amphibians.

**MP039 Acute and Chronic Sulfate Toxicity to Select Freshwater Fish and Aquatic Invertebrates** R. Consbrock, US Geological Survey, CERC, Toxicology; D.K. Hardesty, US Geological Survey; C.G. Ingersoll, USGS, Columbia Environmental Research Center; N. Wang, US Geological Survey, Columbia Environmental Research Center. State water quality standards for sulfate have been developed by Illinois and are being considered by other Midwestern states in the United States. These state standards were developed primarily with acute toxicity data. National acute or chronic water quality criteria have not been developed. The objective of this study was to generate acute and chronic sulfate ( $\text{Na}_2\text{SO}_4$ ) toxicity data to select freshwater fish and aquatic invertebrates for the USEPA to develop the national criteria and for the states to refine the standards for sulfate. Test water were prepared by diluting well water with deionized water to a hardness of about 100 mg/L as  $\text{CaCO}_3$  and a pH of about 8.3 (mg/L: Ca 28, Mg 10, K 1.0, Na 10, Cl 12,  $\text{SO}_4$  19, and dissolved organic carbon < 1.0). Static renewal, acute toxicity tests were conducted with cladoceran (*Ceriodaphnia dubia*; 2-d exposure), midge (*Chironomus dilutus*; 4-d exposure), unionid mussel (*Lampsilis siliquoidea*; 4-d exposure), and fathead minnow (*Pimephales promelas*; 4-d exposure). Chronic toxicity tests were conducted with the cladoceran (7-d static renewal exposure with survival and reproduction endpoints), the midge (up to 41 d flow through exposure with survival, growth, and reproduction endpoints), the mussel and minnow (28- to 34-d flow through exposure with survival and growth endpoints). Preliminary results based on survival endpoint indicated that acute LC50s ranged from about 1,000 to 10,000 mg  $\text{SO}_4$ /L and chronic LC20s ranged from about 400 to 1,000 mg  $\text{SO}_4$ /L for the four tested species. Analyses of growth or reproduction data are ongoing. Further study is needed to evaluate the sensitivity of additional species (e.g., mayflies, oligochaetes) or the potential influence of water quality on the responses of test organisms to sulfate.

**MP040 Physiological Effect of NaCl Exposure on the Larvae of Freshwater Mussels** L. Nogueira, Federal Univ of Rio Grande – FURG, Instituto de Ciências Biológicas; S. Higgins, McMaster Univ; V.L. Loro, Federal Univ of Santa Maria; A. Bianchini, Federal Univ of Rio Grande – FURG; C.M. Wood, McMaster Univ; P.L. Gillis, Environment Canada – Canada Center for Inland Islands. The application and storage of road salts for winter



road maintenance (i.e., de-icing) is a major anthropogenic source of salt to fresh water environments. In Canada, it is estimated that 5 million tons of salt are applied each year. High chloride levels have also been measured in non-winter seasons. In southern Ontario, chloride level of 4 g/L has been reported during spring snow melt. Freshwater mussel larvae (called glochidia) have been shown to be very sensitive to salt, however the toxicological mechanism(s) involved in this sensitivity are unknown. Therefore, our goal was to evaluate physiological effects using environmentally relevant NaCl concentrations on *Lampisilis fasciola* glochidia. Mussel larvae were exposed to NaCl for 48 h. The gradients of exposure were created from the addition of NaCl (0.25 and 1.0 g/L) in the reconstituted hard water (control solution: 0.04 g/L Na and 0.0013 g/L Cl) and also from diluted control solution (0.01 g/L Na and 0.0005 g/L Cl). Following exposure, Na and Cl influx rates and whole body concentration of these ions were assessed as well the ability of the exposed larvae to close their valves, a measure of acute toxicity. Based on the results, glochidia have the ability to maintain Na and Cl influxes and whole body Na and Cl levels relatively unchanged in low exposure concentrations, but these levels increase at high NaCl exposure. The down-regulation of Na uptake in the 1.0 g/L exposure may involve a closing of Na channels. In contrast, glochidia are not able to maintain low Cl influx. The increase in mortality observed when the animals are exposed to high NaCl concentrations may occur due to Na and Cl accumulation and consequently ionoregulatory dysfunction. (Supported by the International Research Chair Program of the International Development Research Centre and the Canada Research Chair Program).

**MP041 Comparative Sensitivity of *Centroptilum triangulifer*, *Ceriodaphnia dubia* and *Daphnia magna* to Standard Salt, TDS and Copper Toxicants** J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; K. Hammer, The McConnell Group c/o USEPA Cincinnati; P. Weaver, The McConnell Group, c/o USEPA; B. Johnson, C. Nietch, USEPA Office of Research and Development; D. Funk, Stroud Water Research Center; D. Buchwalter, North Carolina State Univ. Development of methods for assessing exposure and effects of wastewaters from energy and mineral resource extraction operations on stream invertebrate species is important for establishing protective water quality criteria. *Centroptilum triangulifer* is a parthenogenetic mayfly occurring in depositional habitats of streams and rivers of the Eastern US and Canada. *C. triangulifer* is a relevant insect for monitoring water quality under laboratory and field conditions because of its short life cycle and unique mode of reproduction. In this study, multiple cohorts of *C. triangulifer* was reared using a standardized diet of three diatoms: *Mayamaea atomus* var. *permitis*, *Nitzschia* cf. *pusilla*, and *Achnanthes minutissimum*. Percent survival (80+%), fecundity measurements (1000+) and pre-egg laying weights were used as indicators of overall colony health and fitness over five generations. Comparative testing assessed the exposure sensitivity to  $\text{NaHCO}_3$ , standard salt reference toxicants KCl and NaCl, a metal reference toxicant,  $\text{CuSO}_4$ , currently used in EPA's acute and chronic Whole Effluent Toxicity manuals, and a TDS recipe. Sensitivity tests were run simultaneously with *Ceriodaphnia dubia* and *Daphnia magna* and were conducted for both acute and chronic endpoints. *C. triangulifer* was less sensitive to KCl but 2.8X more sensitive than other culture species to NaCl and  $\text{CuSO}_4$ . Acute  $\text{NaHCO}_3$  results indicated *C. triangulifer* was slightly more sensitive than *C. dubia* and *D. magna*. Chronic results suggested *C. triangulifer* may be 8X more sensitive to NaCl than *C. dubia* and 16X more sensitive than *D. magna*. These and other comparative toxicity results will be presented.

**MP042 Methods Development for a Spatially Explicit Population-level Risk Assessment, Uncertainty Analyses, and Comparison with Risk Quotient Approaches** S. Raimondo, United States Environmental Protection Agency, Gulf Ecology Division, National Health and Environmental Effects Laboratory, US Environmental Protection Agency, Gulf Ecology Division; J. Awkerman, US Environmental Protection Agency, Gulf Ecology Division; B. Hemmer, US Environmental Protection Agency; M.G. Barron, USEPA, Gulf Ecology Division. The standard framework of Ecological Risk Assessment (ERA) uses organism-level assessment endpoints to qualitatively determine the risk to populations. While organism-level toxicity data provide the pathway by which a species may be affected by a chemical stressor, they neither indicate the probability that an effect (i.e., reduced survival, reduced reproduction) will impact the population in a natural system, nor

the magnitude of that impact. Population models have the potential to be used in risk characterization; however they are not applied in ERA because uncertainty analyses and minimum data requirements are unknown. To quantify uncertainties in risk estimation (e.g., confidence in parameter estimation), we will apply spatially explicit population models of the sheepshead minnow (*Cyprinodon variegatus*) in a case study risk assessment of the Deepwater Horizon Oil Spill in Barataria Bay, LA. Sediment samples were collected throughout Barataria Bay in the fall of 2010 and will provide an interpolated, quantitative layer of PAH exposure to estuarine organisms. We will conduct full life cycle sediment toxicity tests to obtain chronic dose response functions for the sheepshead minnow exposed to PAHs. Exposure and concentration response layers will be applied to a spatially-explicit, density dependent sheepshead minnow population model. We will conduct several levels of ERA of varying complexity, including a traditional ERA using standard risk quotient based approaches, a simple non-spatial population-level ERA, and spatial population-level ERAs of varying complexity. Uncertainty analyses of each ERA will identify the relationship between ERA complexity, uncertainty, and data requirements.

**MP043 Biological Community Monitoring to Evaluate Pulp and Paper Discharge Relocation Success and Effects in the Lower St. Johns River Basin, FL** O. Burgess, Univ of Florida, Fisheries and aquatic Sciences, Environmental Resource Consultants; T. Gross, Environmental Resource Consulting, Dr. Timothy S. Gross, Univ of Florida; S. Holm, Georgia-Pacific Corporation, Environmental Affairs. Long-term (1999-2008) assessments of Georgia Pacific's Palatka Mill and biological effects have demonstrated that mill upgrades resulted in reduced biological responses. Results indicate that "masculinization" responses in *Gambusia* are not an appropriate model for assessing effects, while largemouth bass are a viable model to assess effects. The final stage in these long-term mill upgrades will be the relocation of discharge from Rice Creek to the St. Johns River. To evaluate the success and potential biological effects of this relocation a "Plan-of-Study" has been developed focusing on biological community monitoring for two years pre and post relocation. Biological community assessments include evaluations across multiple trophic levels: algae, plankton (phyto and zoo), submerged aquatic vegetation, macroinvertebrates, and fish. Efforts also include a long-term utilization of largemouth bass to assess reproductive, endocrine and health effects. Community assessments focus on levels of biological organization above the organism while evaluations of largemouth bass focus on levels at the organism and below. These efforts are among the first to utilize and apply NCASI's Large River Monitoring Program success and procedures to an evaluation of mill discharge relocation. Efforts have required modification of standard procedures which were developed for Wadeable streams. It has also been critical to assess seasonal and annual variance and characterize habitat variance which must both be exceeded to detect effects. Pre-planned comparisons have been established for subsequent analyses. Results indicate similar biological diversity across the 20 km study range (10 km north and south of proposed discharge relocation site). Higher trophic levels have greater diversity due to co-occurrence of marine and freshwater species. Significant effects of record winter cold temperatures, drought, storm events and algal blooms have indicated effects at the community level. Evaluations of biological community effects are likely a critical component in a final assessment of mill discharge relocation, especially when paired with assessments of organism effects.

**MP044 Investigation of Estrogenic Effects of Bifenthrin in Rainbow Trout, *Oncorhynchus mykiss*** N. Riar, D. Schlenk, Univ of California, Environmental Toxicology. Pyrethroids are a commonly used class of pesticide in California in both agricultural and urban applications. Although pyrethroids are reported as having nominal effects in mammals, they have been shown to have toxic effects in aquatic organisms, especially invertebrates and fish. Their persistence in waterways due to their multitude of uses, require that chronic exposure and effect must be investigated. This study examines Bifenthrin, a third generation pyrethroid that has been seen in levels as high as 73 ng/L in northern California waterways. It is similar to another pyrethroid, Permethrin, which has been shown to have estrogenic activity at concentrations as low as 10 ug/L. Exposures were performed with Rainbow trout (*Oncorhynchus mykiss*) for 10 days at 0, 10, 50, and 150 ng/L. At the end of the experiment, plasma was collected and vitellogenin protein was semi-quantified using Western Blots. Preliminary data showed 33, 29, 40, and 42 ng vitellogenin per mg protein in fish exposed to 0, 10, 50, and 150 ng/L concentrations respectively. This suggests that Bifenthrin



and/or its metabolites result in estrogenic activity in Rainbow trout. Further studies will isolate the specific source of estrogenicity. Also, additional factors that may further enhance these effects, including increased salinity in the exposure media, will also be investigated. The results of this study will aid in understanding the synergistic effects of global climate change and low concentrations of persistent pesticides. Such information is essential for understanding the chronic toxicity to important salmonid species in the delicate estuarine ecosystems of northern California.

**MP045 Changes in a Common Biomarker (Vitellogenin) Do Not Correlate to Higher-level Reproductive Responses in Mummichog Exposed to 17 $\alpha$ -ethynylestradiol** T. Bosker, Univ of New Brunswick, Canadian Rivers Institute; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology; A. Lister, Wilfrid Laurier Univ; D. MacLachy, Wilfrid Laurier Univ, Canadian Rivers Institute, Wilfrid Laurier Univ, Dept of Biology. A recent meta-analysis on short-term reproductive tests demonstrated that there are quantitative relationships between commonly-used biomarkers and egg production, a functional endpoint which can be linked to population-level effects. These relationships were found regardless of fish species or contaminant mode of action. In the current study we investigated the response of mummichog (*Fundulus heteroclitus*), a small-bodied estuarine fish species found along the Atlantic coast of North-America, when exposed to the potent estrogen 17 $\alpha$ -ethynylestradiol (EE<sub>2</sub>). Fish were exposed in three experiments in a flow-through system to nominal concentrations ranging from 0ng/L to 3,000ng/L EE<sub>2</sub>. In the first two experiments, fish were exposed to a concentration series of EE<sub>2</sub> to determine the effect on relative gonad size and functional endpoints. No changes in gonad size or egg production were found even when mummichog were exposed to very high levels of EE<sub>2</sub> (up to 3,000ng/L) for prolonged periods of time (28d). However, low sample size and high variance resulted in low power to make conclusions. In the third experiment, fish were exposed to 100ng/L EE<sub>2</sub> using an optimized adult fish reproductive test study design. No effects of EE<sub>2</sub> on gonad size or egg production were observed while a >35-fold increase in hepatic vitellogenin gene expression ( $p < 0.001$ ) was found. This study demonstrates that fish exposed to high levels of a potent estrogenic compound can respond to a common biomarker without correlating responses in functional endpoints. It is important to be cautious in linking biomarker responses to functional responses without strong species-specific data to support the connection.

**MP046 Environmental Influences on the Accumulation of Ethynylestradiol in Marine Teleosts** T. Blewett, D. MacLachy, Wilfrid Laurier Univ, Canadian Rivers Institute, Wilfrid Laurier Univ, Dept of Biology; C. Wood, McMaster Univ, Dept of Biology. The synthetic estrogen 17- $\alpha$ -ethynylestradiol (EE<sub>2</sub>), an endocrine disruptor originating from birth control and hormone replacement therapy, is discharged in wastewater treatment plant (WWTP) effluents. The present study employed radio-labeled EE<sub>2</sub> to examine the impact of temperature and salinity on the uptake of EE<sub>2</sub> in male killifish (*Fundulus heteroclitus*). Fish were exposed to a nominal concentration of 100 ng/L EE<sub>2</sub> for 2 h. Actual concentrations were lower due to EE<sub>2</sub> adsorption to the exposure system, but uptake rates were normalized to 100 ng/L. Oxygen consumption rates (MO<sub>2</sub>), whole body EE<sub>2</sub> uptake rates, and tissue-specific EE<sub>2</sub> distribution were monitored. EE<sub>2</sub> uptake by freshly killed fish was negligible. In killifish acclimated to 18°C at 16 ppt (50 % seawater), MO<sub>2</sub> and EE<sub>2</sub> uptake were both much lower after 24-h exposure to 10°C and 4°C, and increased after 24-h exposure to 26°C. Transfer of killifish to fresh water for 24-h tended to lower EE<sub>2</sub> uptake rate, and long-term acclimation to fresh water reduced it by about 70 %. Long-term acclimation to 100 % sea water (32 ppt) also reduced EE<sub>2</sub> uptake rate by about 50 % relative to 16 ppt. However, this was not seen in rainbow trout (*Oncorhynchus mykiss*) where uptake rates were the same in FW- and 16 ppt-acclimated trout. The tissue-specific accumulation of EE<sub>2</sub> was found to be the highest (40-60 % of the total) in the liver plus gall bladder across all exposures, and the great majority of this was in the bile in killifish, regardless of temperature or salinity, whereas in trout accumulation was the highest in the carcass at 70 % of the total. The carcass was the next highest accumulator (30-40 %) in killifish, followed by the gut (10-20 %) with only small amounts in gills and spleen. Drinking rate, measured with radio-labeled polyethylene glycol-4000, was about 25-times greater in 16 ppt-acclimated killifish relative to freshwater-acclimated animals. However, drinking accounted for less than 30 % of gut accumulation, and therefore a negligible percentage of whole body EE<sub>2</sub> uptake rates. In general, there were

strong positive relationships between EE<sub>2</sub> uptake rates and MO<sub>2</sub>, suggesting similar pathways for uptake across the gills of these lipophilic molecules. These data will be useful in developing a predictive model of how variations in key environmental parameters (salinity, temperature, dissolved oxygen) affect EE<sub>2</sub> uptake in estuarine fish, to determine optimal timing and location of WWTP discharges.

**MP047 Understanding Effects of Salinity on 17 $\alpha$ -ethynylestradiol (EE<sub>2</sub>) Uptake and Effects in Mummichog (*Fundulus heteroclitus*)** D. MacLachy, Wilfrid Laurier Univ, Canadian Rivers Institute, Wilfrid Laurier Univ, Dept of Biology; T. Blewett, McMaster Univ, Dept of Biology; T. Bosker, Univ of New Brunswick, Canadian Rivers Institute; E. Gilio Meina, Wilfrid Laurier Univ, Canadian Rivers Institute; T. Nadon, A. Lister, Wilfrid Laurier Univ; L. Bragg, Univ of Waterloo; S. Currie, Mount Allison Univ, Dept of Biology; K. Munkittrick, Univ of New Brunswick; M. Servos, Univ of Waterloo; C. Wood, McMaster Univ. We have undertaken a series of experiments exposing the mummichog or killifish (*Fundulus heteroclitus*) to 17 $\alpha$ -ethynylestradiol (EE<sub>2</sub>) at a range of concentrations and salinities. The overall goals of the experiments are to elucidate whether salinity of exposure impacts EE<sub>2</sub> uptake and/or effects in this small-bodied, euryhaline fish species. Compared to other model fish species tested, mummichog have a high resistance to the endocrine-disrupting effects of environmentally-relevant concentrations of EE<sub>2</sub>. EE<sub>2</sub> uptake in mummichog significantly changed with salinity (0ppt < 32ppt < 16ppt) with the highest EE<sub>2</sub> accumulation (60%) in the liver (including gallbladder). Over repeated exposures of 14-28 days, no effects of EE<sub>2</sub> on gonad size or egg production were observed while increases in hepatic vitellogenin (vtg) induction were consistently found. Vtg induction in male mummichog was approximately 10-fold greater in fish exposed to EE<sub>2</sub> at 16ppt salinity compared to fresh water ( $p < 0.001$ ). Regardless of salinity (0, 16, 32 ppt), use of steroidogenic precursors in vitro ovarian incubations decreased 17 $\beta$ -estradiol production at various points in the steroidogenic pathway before, at and downstream of cholesterol conversion to pregnenolone. Increases in ovarian testosterone production in vitro were variable and indicate decreases in aromatase could be a dominant factor affecting 17 $\beta$ -estradiol production. Overall, salinity does not appear to be influencing effects of EE<sub>2</sub> on reproduction but does affect uptake and some molecular- and tissue-level endocrine responses. The high degree of EE<sub>2</sub> accumulation in the liver and bile could indicate that metabolism and clearance participate in the resistance of mummichog to environmentally-relevant levels of EE<sub>2</sub>. Studies to understand the interactions of temperature, salinity, uptake, metabolism and effects are underway.

**MP048 Development of a Complete Life Cycle Sediment Toxicity Test for the Sheepshead Minnow (*Cyprinodon variegatus*)** E. Degner, Gustavus Adolphus College; B. Hemmer, US Environmental Protection Agency; H. Rutter, Student Services Contractor to USEPA; C.R. Jackson, US Environmental Protection Agency; M.G. Barron, USEPA, Gulf Ecology Division; S. Raimondo, United States Environmental Protection Agency, Gulf Ecology Division, National Health and Environmental Effects Laboratory, US Environmental Protection Agency, Gulf Ecology Division. Existing sediment toxicity test methods are limited to acute and chronic exposure of invertebrates and acute exposure of vertebrates, with limited guidance on the chronic exposure of vertebrates, specifically fishes. A series of life stage-specific studies were conducted to determine optimal test conditions for exposing the sheepshead minnow (*Cyprinodon variegatus*) to contaminated sediment throughout its complete life cycle. Specifically, these methods will be used to expose fish to oil-contaminated sediment collected from Barataria Bay, LA. Reference sediment used in these studies was collected from a marsh habitat in Choctawhatchee Bay, FL and had similar grain size characteristics as Barataria Bay. The hatching success of the egg stage was tested in well plates of various sizes (6, 12, 24 wells) with various amounts of sediment under stagnant and agitated conditions. Test chambers for larvae, juvenile and adult stages were developed to accommodate dissolved oxygen and fish density demands, minimize ammonia accumulation, and represent realistic estuarine exposure conditions. Larvae were exposed in static conditions, while studies were conducted to compare static and flow through exposure for juveniles and adults. Comparisons among flow through and static conditions included flow rates and types (pulse, continuous). Studies were also conducted to develop methods to collect spawned eggs from test chambers to determine hatch success of next generation embryos. These test development methods can be used as guidance for future life cycle sediment exposure studies involving fishes.

**MP049 Waterborne Zinc Effects at Different Salinities on Oxidative Stress Parameters and Antioxidant Response of the Killifish (*Fundulus heteroclitus*)** V. Loro, McMaster Univ, Biology Dept; C. Wood, McMaster Univ, Dept of Biology. We evaluated the effect of waterborne zinc exposure at different salinities on oxidative stress parameters and antioxidant response in *Fundulus heteroclitus*. Killifish were exposed to 500  $\mu\text{g/L}$  of zinc (96h) at 0‰, 10‰, 30‰ and 100‰ sea water, considering 35 ppt as 100‰. Fish exposed to zinc at salinities of 0‰ and 10‰ had increased reactive oxygen species (ROS) in gill, liver, intestine and muscle tissues. However exposure to zinc at 30 and 100‰ salinities resulted in ROS levels similar to control. The total oxidative scavenging capacity (TOSC) was quantified. For gill liver and intestine exposed to zinc and salinities of 0‰ and 10‰, TOSC was decreased. However fish exposed to zinc and salinities of 30‰ and 100‰ recovered TOSC. Muscle tissue showed TOSC decreased for zinc exposure at 0‰ sea water; the other salinity groups and zinc exposure showed levels of TOSC similar to control. Regarding the antioxidant enzymes, superoxide dismutase (SOD) activity was reduced only in the group exposed to zinc 0‰ sea water. Increased SOD was observed in zinc exposed fish at salinities of 30 and 100‰. The activity of catalase showed reduce activity in zinc exposed fish at salinity 0‰ and no change in other salinities. Glutathione-S-transferase (GST) activity increased in all fish tissues exposed to zinc at salinities (10% until 100%). Only at 0‰ salinity did zinc exposed fish exhibit reduced activity in liver, intestine and muscle. Glutathione (GSH) showed reduced levels for all tissues tested in fish exposed to zinc at 0‰ salinity. The other salinities exposed to zinc increased this parameter. Our results improve understanding of zinc toxicity in estuarine fish, and highlight the protective role of higher salinities in ameliorating the oxidative stress associated with zinc toxicity. (supported by IRC program of IDRC/CRC (Canada); NSERC CRD Program, ILZRO, ICA, CDA, NiPERA, Teck, & Vale)

**MP050 HSP70 Expression as a Biologically Significant Biomarker of Fish Health Under Field Conditions** D. Webb, Curtin Univ, Environment and Agriculture; M. Gagnon, Curtin Univ, Dept of Environment and Agriculture, Curtin Univ (Bentley Campus), Dept of Environment and Agriculture, Curtin Univ, Environment and Agriculture. Heat shock protein 70 (HSP70) levels in three tissue types (gill, liver and muscle) from black bream (*Acanthopagrus butcheri*) collected in a highly variable estuarine environment were measured to determine which tissue provides better inter-site discrimination. The usefulness of hsp70 expression to identify anthropogenic stress under field conditions was evaluated. Inter-site differences were detected in HSP70 levels in gill and white muscle of black bream while liver showed no spatial difference. There was high inter-fish variability in HSP70 levels in each tissue group. A post-hoc power analysis of the data sets for each tissue found that in black bream, white muscle provided the best discriminatory power to elucidate spatial variability. Only 11 fish are needed to be sampled under field conditions to identify inter-site differences when using white muscle tissue to obtain a power of 80%, while gill tissue requires at least 14 fish to be sampled and liver needs over 20 fish to reduce the chance of a type II error. Due to a high variability observed between tissues and between individuals, field measurement of hsp70 should be complemented by evidence of changes to metabolic enzyme activity, DNA damage, and/or blood cortisol levels. As part of a suite of measurements, hsp70 expression has some value as a biomarker of fish health.

**MP051 Comparative Study of Reproductive Capacity and Oxidative Stress in Blueback Herring in North Carolina** P. McClellan-Green, North Carolina State Univ, Ctr for Marine Sciences & Technol. Recent stock assessments by the North Carolina Division of Marine Fisheries (NCD-MF) for blueback river herring has established the species as depleted in northeastern North Carolina. Data collected by NCDMF indicate that the spawning stock biomass of bluebacks are declining, that juvenile production in the river system is low and that the mean body length compared to age for the stock is significantly reduced. While each of these factors is related to over harvesting of the population they can also be indicative of anthropogenic impacts on the reproductive physiology of the species. Environmental and anthropogenic factors such as temperature, oxygen levels and pollution can increase the stress experienced by fish inhabiting these environments and thus affect their reproductive capacity (or fecundity). In 2010, a study initiated examining the temporal changes in reproductive potential and oxidative stress of blueback river herring in the Chowan River. Blueback herring were collected each week during their spawning season for a total of 8 weeks. Analysis of physiological parameters indicate their reproductive potential

is highest during the initial three weeks of spawning and decreases as the season continues. Levels of steroid hormone, 11-ketotestosterone and estradiol, were also monitored on a weekly basis. Reproductive parameters were then compared to levels of oxidative stress, for example glutathione, LPO and catalase activity. Our results indicate that oxidative stress increases up to the peak spawning period and then declines as the fish enter their regenerative phase. These results could have distinct implications for monitoring of oxidative stress in field studies.

**MP052 Acute Effects of Aniline on Two Estuarine Species, *Palaemon serratus* and *Pomatoschistus microps*: Effects on Biomarkers and Swimming Velocity** L. Luis, Univ of Porto, CIIMAR & ICBAS; L. Guilhermino, Univ of Porto, CIIMAR & ICBAS, Laboratory of Ecotoxicology, Univ of Porto, ICBAS & CIIMAR, Universidade do Porto. Aniline is one of the Hazardous and Noxious Substances (HNS) transported at highest amounts by sea and thus concerns existing regarding potential accidents resulting in spillages of this substance in the marine environment. It is also commonly used in several industrial activities and thus is an environmental contaminant in several regions, including estuaries. Since the existing information on its toxic effects on estuarine organisms is still scarce, more knowledge is needed particularly on benthic species with an ecologically important function. Thus, to contribute to the question, the acute effects of aniline on two benthic species with ecologically relevant functions in a considerable number of European estuaries were investigated. For each species, 96h semi-static bioassays were carried out with animals being exposed individually to aniline through water in temperature and photoperiod controlled rooms. At the end of the exposure period, swimming velocity and several biomarkers were determined. Aniline was found to be able to decrease swimming velocity of animals and to induce changes in several biomarkers. The ecological implications of these findings are discussed. This study was done in the scope of the project "RAMOCS – Implementation of risk assessment methodologies for oil and chemical spills in the European Marine Environment" (ERA-AMPERA/0001/2007), funded by the Portuguese Foundation for the Science and the Technology (FCT) and FEDER funds in the framework of the EU AMPERA ERA-NET (ERAC-CT2005-016165). L. Luís has a grant from FCT (SFRH/BI/51043/2010).

**MP053 Histological Studies in Fish for the Monitoring of POPs in Mexico** X. Guzman-Garcia, I. Hernandez-Calderas, C. Garcia Gonzalez, V. Ramirez Trejo, J.E. Torres Padilla, J.G. Trejo Ramirez, Universidad Autonoma Metropolitana, Hidrobiologia; P. Ramirez Romero, Universidad Autonoma Metropolitana, Hidrobiologia, U.A.M. Iztapalapa, Depto. De Hidrobiologia. Environmental monitoring of POPs is mostly based on chemical concentrations in the different environmental compartments, including organisms. However, these measurements can't provide information regarding bioavailability and effects of these compounds, therefore the National Monitoring Program (PRONAME by its Spanish acronym) decided to include histopathology analysis in fishes. The objective of this work is to present the results of the comparison of three PRONAME sites: Yaqui Valley in Sonora (with a strong agricultural influence), Manantlán in Jalisco and Celestún in the Yucatan Peninsula (this last two as reference sites). Samples of carps, mullets and cat fish were collected at the PRONAME sites and brought to the laboratory for histological analysis. Liver, pancreas and intestine tissues were processed in Leica instruments (tissue processor TP 120, inclusion center EG1140H coupled with a chillin plaque EG1140C), three serial 5  $\mu\text{m}$  cuts were obtained with a microtome and stretched in a 50 oC bath. These were HE stained and analyzed under optical microscope. Results showed that carps from Manantlan, a Biosphere Reserve, have few lesions; however catfish from Celestun presented the highest incidence of lesion of the three sites, which was an unexpected results since this site is also a Biosphere Reserve, however this result agreed with chemical analysis, in the sense that more POPs have been reported for this site. Sonora mullets presented an intermediate incidence of lesions in spite of having been captured in coastal water bodies that receive the drainage of the agricultural area.

**MP054 Temporal Trend Analysis of Polychlorinated Biphenyls and Organochlorine Pesticides in the Great Lake Fish, 1999-2009** F. Chang, Clarkson University, Civil and Environmental Engineering; T.M. Holsen, Clarkson Univ; P.K. Hopke, Clarkson Univ, Dept of Chemical Engineering; B. Crimmins, Clarkson Univ; J.J. Pagano, State Univ of New York-Oswego, Environmental Research Center; M.S. Milligan, SUNY Fredonia, Dept of Chemistry. Temporal trend analysis of the newest Great Lake Fish



Monitoring and Surveillance Program (GLFMSP) data showed a statistically significant decrease of persistent bioaccumulative toxic contaminants in Lakes Huron, Ontario, and Michigan over the 1999-2009 period. In contrast, trend analysis showed only minor fluctuations or relatively stable levels in Lakes Superior and Erie during the same period. For the three lakes showing a decrease the average annual decrease of total polychlorinated biphenyl (PCBs) concentrations was  $5.0 \pm 2.6\%$ . Dichloro-diphenyl-trichlorethane and its metabolites (DDTs), dieldrin, and other organochlorine pesticides showed faster decreases, ranging from  $10 \pm 4.3\%$  to  $20 \pm 7.1\%$  per year. With the exception of PCB levels, this current decrease was greater than was shown by an earlier trends analysis which estimated an annual contaminant decrease of about 2-5% for the 1980-2003 period. Our trend analysis of PCBs decrease in Great Lakes fish ranging from 3-8% per year in 1999-2009 agrees well with the reported 3-10% decrease per year in other Great Lakes environmental media. Additionally, characteristics of PCB congener profiles were analyzed within lakes and among lakes fish samples to explore PCBs sources to Great Lakes fish.

**MP055 Monitoring for Hydrophobic Contaminants in Urban-impacted Nearshore Waters of the Great Lakes Using Polyethylene Passive Samplers**

**P.A. Helm**, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; A. Muscalu, E. Reiner, L. Shen, T. Kolic, A. Tencic, A. Boden, K. MacPherson, D. Morse, Ontario Ministry of the Environment. Urban areas are significant contributors of environmental contaminants to adjacent waterways through atmospheric contributions, direct stormwater discharge, urban tributary inputs, and municipal wastewater discharges. The wide variety of chemicals present in these areas represent both the legacy of past-use of persistent, bioaccumulative and toxic industrial chemicals and pesticides, such as polychlorinated (PCBs) and organochlorine pesticides (OCPs), and of current-use chemicals related to use in consumer products, such as flame retardants, and continued combustion by-product compounds like polycyclic aromatic hydrocarbons (PAHs). In this study, passive polyethylene (PE) samplers were deployed in urban streams in the Toronto, Ontario area and in Lake Ontario in nearshore waters adjacent to urban areas from Hamilton Harbour in the west end of the lake to waters off Pickering, Ontario, east of Toronto. Lake sites included those influenced by municipal wastewater discharges and long term nearshore monitoring sites. PE sampler extracts were subjected to multi-dimensional gas chromatography (GCxGC)-electron capture detection (ECD), in addition to analyses for PAHs, polybrominated diphenylether (PBDE) and non-PBDE flame retardants. GCxGC-ECD provides excellent separation of target analytes with minimal coelution, and can also be used for analytical triage to screen for additional halogenated compounds. The GCxGC screening indicated the presence of a considerable number of compound classes at several stations, including chlorinated paraffins, PBDEs, chlorinated dibenzo-*p*-dioxins and dibenzofurans, as well as several unknown halogenated substances. The greatest concentrations, and number and diversity of substances, were observed in PE samplers deployed at locations more influenced by municipal wastewater and urban/industrial development, such as Etobicoke Creek and the Don River in Toronto, and Hamilton Harbour. In addition to presenting 2D chromatograms, the presentation will highlight estimated concentrations for legacy and current-use compounds.

**MP056 Bioaccumulation of Selected Halogenated Organic Flame Retardants in Remote Lakes and in the Great Lakes**

**D. Muir**, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute; C. Teixeira, J. Epp, T. Young, X. Wang, M. Keir, S. Backus, Environment Canada. In this study the bioaccumulation and concentrations in surface waters of a wide range of non-legacy halogenated organic compounds (HOCs) was determined in remote lakes within the Canadian shield and in the open Great Lakes. Large volume samples of surface waters (100 L) were collected from two remote lakes Lake Opeongo (Algonquin Provincial Park, ON) and Siskiwit Lake (Isle Royale National Park, MI) as well as from surface waters of the lower Great Lakes over the period 2005-2010. Zooplankton (>100 µm), mysids, forage fish and lake trout were obtained from Lakes Erie, Ontario and Opeongo while only lake trout were available from Siskiwit Lake. Extracts were screened for 27 individual BDEs (Br3-Br10) and 20 Br3-Br6 compounds/ PBDE replacements and other HOCs using GC-electron capture negative ion mass spectrometry (GC-EC NIMS) with a HP-5MS and RTX-1614 capillary columns. Br3-Br6 compounds and Dechlorane Plus

(DP) were non-detect (< 0.1 pg/L) in Siskiwit and near detection limits in Opeongo lake waters. Pentabromo-ethylbenzene (PBEB), 1,3,5-tribromo-2-methoxy-4-methylbenzene (Br3MeBz), allyl 2,4,6-tribromophenyl ether (ATE) and dibromopropyl 2,4,6-tribromophenyl ether (DPTE) were the most prominent non-PBDE HOCs in Opeongo lake waters; present at sub-pg/L concentrations. A larger suite of HOCs were detectable in Lake Ontario waters including DP, Br3MeBz, PBEB, DPTE, 12345-pentabromobenzene (Br5Bz), penta- and hexabromotoluene, and 2-ethyl-1-hexyl 2,3,4,5-tetrabromobenzoate (EHTeBB) although concentrations were near or at MDLs. The tris-chloroalkyl phosphates (TCEP, TCP, TDCP) were present at 5-50 ng/L in central Lake Ontario waters. A wide range of Br3-Br6 compounds were detected in zooplankton from Lake Ontario including Br3MeBz, 1,3,5-tribromobenzene (TBB), tetrabromoxylene (TBX), bis(tribromophenoxy)ethane (BTBPE), PBEB, and BPTE. Lake Opeongo zooplankton had a more limited suite, with Br3MeBz, ATE and Br5Bz present at low pg/g (wet wt) concentrations. BDE47, BDE153 and Br3MeBz had the highest trophic magnification factors based on the slope of the log (lipid wt) concentrations versus trophic level. TBX, 1,3,5-TBB and BTBPE showed trophic dilution while other compounds e.g., BDE202 (and other hepta- and octaBDEs) were detectable in most samples but showed limited increases with trophic level.

**MP057 Organophosphate Flame Retardants and Benzothiazoles in Open Waters and Tributaries of the Great Lakes**

**D. Muir**, Environment Canada, Aquatic Ecosystem Protection Research Division, National Water Research Institute; C. Teixeira, A. Sett, B. Lee, T. Peart, Environment Canada. Triaryl/alkyl phosphates/tris(haloalkyl) phosphates (TAAPs/THAPs) and benzothiazoles (BTZs) are of interest as contaminants in Great Lakes waters because they are high production volume chemicals with relatively high log K<sub>ow</sub>s and molecular structure indicative of slow biodegradation. Production of TAAP/THAPs is reported to have increased by 11% in the USA and 57% in Asia from 2005 to 2008 due to their use as replacements for the brominated flame retardants. BTZs are widely used as corrosion inhibitors, and in aircraft deicing fluids and as UV absorbers in polymers. All 3 classes are thus directly emitted to the environment via volatilization and abrasive losses from polymers and indirectly via poor removal in waste water treatment. TAAP/THAPs were extracted from 1L water samples using solid phase cartridges and then analysed by LC-MS/MS using positive Electron Spray Ionization (ESI+). Nine TAAP/THAPs were found in sewage treatment plant influent and effluent samples at low ng/L to mid µg/L levels, with tris-butoxyethyl-phosphate (TBEP) and tris-chloroalkyl phosphates (TCEP, TCP, TDCP) present at highest concentrations. TBEP, TCEP, TCP, and TDCP were also the major TAAP/THAPs at the 3 mid lake sites in of Lake Ontario (west, central and eastern basins) at

**MP058 Perfluoroalkylated Compounds (PFCs) in Great Lakes Fish as Part of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP)**

**B. Crimmins**, X. Xia, P. Hopke, Clarkson Univ; M. Milligan, SUNY Fredonia; J. Pagano, SUNY Oswego; T. Holsen, Clarkson Univ. The Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) was recently tasked with exploring non-legacy contaminant distributions in the Great Lakes' top predators. Perfluoroalkylated compounds are one such class of compounds and have received a significant amount of attention in the past decade. Previous quantifications have been hindered by interferences, ion suppression and lack of comprehensive quantitation and recovery standards. Interlaboratory comparisons have also shown significant variability among laboratories and procedures. While there is typically a trade-off between instrument sensitivity, specificity and quantitative linearity we have developed a method for PFCs using newly developed UPLC-QTOF technology addressing each of these concerns. The current platform describes the methodology, instrumentation specifications and performance evaluation of this method on Great Lakes' biota. In addition, concentrations in lake trout and walleye (Lake Erie) collected from 2004-2009 will be presented with an emphasis on spatial and temporal trends.

**MP059 The Relative Sensitivity of Macrophyte and Algal Species to Herbicides and Fungicides: An Analysis Using Species Sensitivity Distributions**

**J. Giddings**, Compliance Services International. In January 2008, at a SETAC workshop on Aquatic Macrophyte Risk Assessment for Pesticides (AMRAP), a Species Sensitivity Distribution (SSD) working group was formed to address uncertainties about the sensitivity of *Lemna* and other standard test species to pesticides relative to other aquatic macrophyte



species. For 11 herbicides and 3 fungicides for which relevant and reliable data were found for at least 6 macrophyte species (considered the minimum for SSD analysis), SSDs were fitted using lognormal regression. The position of *L. gibba* in each SSD was determined. The sensitivity of standard algal test species relative to the macrophytes in each SSD was also considered (algae were not included in the SSD). In recognition of the known sensitivity of *Myriophyllum* species to some herbicides and ongoing activities to develop standardized test methods for these species, the position of *M. spicatum* in each SSD was also determined where data were available. Results indicated that *L. gibba* is among the most sensitive macrophyte species for approximately half of the chemicals examined. In the majority of cases, the lowest standard algal test species endpoint was lower than the most sensitive macrophyte endpoint. *M. spicatum* was among the most sensitive macrophytes for approximately one-quarter of the chemicals. While no single species consistently represents the most sensitive aquatic plant species, for 12 out of 14 compounds algae and *L. gibba* include an endpoint that is near or below the 5th percentile of the macrophyte SSD. For the other two compounds, *M. spicatum* is the most sensitive species of all aquatic plants considered. The AMRAP SSD workgroup includes Stefania Loutseti (DuPont, workgroup chair), Gertie Arts (Alterra), Nina Cedergreen (U. Copenhagen), Heino Christl (APC), Jo Davies (Syngenta), Michael Dobbs (Bayer CropScience), Mark Hanson (U. Manitoba), Udo Hommen (Fraunhofer Institute), Joy Honegger (Monsanto), Phil Manson (Cheminova), Giovanna Meregalli (Dow AgroSciences), and Gabe Weyman (Makhteshim-Agan).

**MP060 Is the Sensitivity of Lemna and Lemna Endpoints to Metsulfuron-methyl Representative of Other Macrophyte Species and Endpoints?**

G.H. Arts, Alterra Wageningen Univ and Research Centre, Centre for Water and Climate, Environmental Risk Assessment; A. Jonas, E. Dorsman, Alterra Wageningen Univ and Research Centre. Is the sensitivity of Lemna and Lemna endpoints to metsulfuron-methyl representative of other macrophyte species and endpoints? To investigate this topic, toxicity values were collected from laboratory tests with a range of macrophyte species (incl. Lemnaceae) and from literature. Laboratory experiments followed test methods under non-axenic, controlled conditions (21 days; 17°C; 14/10 light/dark regime; 400 W Philips HPI-T lamps; illumination  $160 \pm 65 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at water surface level; 1.5 L glass test vessels; Barko and Smart growth medium; fine clay sediment; 3 apical, unbranched macrophyte shoots (10 cm) or 10 fronds of Lemnaceae; treatments 1 – 3300 ng/L metsulfuron-methyl). We plotted the resulting toxicity data and calculated log-normal distributions: I. Endpoint EC50 distributions of Lemnaceae at days 4, 7, 14, 21. II. Lognormal distributions including all macrophyte EC50 values. III. Lognormal distributions of all macrophyte EC50 values excluding the Lemnaceae toxicity values. The results show that despite the non-axenic nature of the tests, the sensitivity and variability of Lemna endpoints fitted well within published ranges. Variability is only larger at day 7. When considering all macrophyte EC50 values including Lemnaceae toxicity values, the distribution did not pass the Anderson-Darling goodness-of-fit test. However, when excluding the Lemnaceae toxicity values, the data did pass this test and fitted a log-normal distribution. This suggests that the distribution of toxicity values from Lemnaceae does not fit the distribution of toxicity values from submerged aquatic macrophytes. The range of macrophyte sensitivity is rather broad, with a 100 to 1000-fold difference between the most sensitive and least sensitive species and endpoint. Resulting HC5 values from distributions including all macrophyte endpoints are lower than published ones. HC5 values from distributions with Lemnaceae are within the ranges of published values. The representativity of Lemna and Lemna endpoints of other macrophyte species and endpoints very much depends on the endpoint considered. Root and new shoot endpoints are very sensitive endpoints for submerged aquatic macrophytes. Specific leaf area is a very sensitive endpoint for Lemnaceae.

**MP061 The Little Plant that Could: 7 Years of Effluent Testing at Canadian Metal Mines** L. Taylor, Environment Canada; R. Scroggins, Environment Canada, Ecotoxicology and Wildlife Health Division. Under the 2002 Canadian Metal Mining Effluent Regulations (MMER), there is a requirement to conduct an Environmental Effects Monitoring (EEM) program to evaluate the effects of mine effluent on local fish species, fish habitat and uses of fisheries resources. As part of this program, sublethal toxicity tests were conducted twice per year for the first three years for regulated mines and once per year in subsequent years. For a final discharge point at a mine, effluent testing needs to be conducted using a fish, an

invertebrate, an aquatic higher plant and an algal species. The purpose of sublethal toxicity (SLT) testing in this context is to answer the question: “is there evidence from sublethal toxicity testing that mine effluent may affect fish, invertebrates and aquatic plants?” Specifically, effects data using standardized methods would provide an indication of the degree of variability in effluent quality and temporal trends. The Environment Canada biological test method for assessing the effects on macrophytes uses *Lemna minor* (EPS 1/RM/37) as the representative aquatic higher plant species. Toxicity data collected over 7 years (2003-2009) was reviewed for mines across Canada. A total of 660 individual *Lemna* tests revealed that an inhibition of growth (as seen by a reduction in frond number) is a sensitive endpoint for metal mine effluents; whereas the reduction in growth based on dry weight was less so. An improvement in effluent quality across Canada was evident from the first three years of monitoring (2003-2005) to the second three years (2006-2009), where the number of tests reported to have no effect (IC25 >100% or undiluted effluent) increased from 27 to 41%. The greatest improvement was seen with iron ore mines, followed by uranium and precious metal (e.g., Ag, Au, Pd) mines. Relatively small improvements were seen with base metal (e.g., Cu, Zn, Ni) and other mine types. An investigation was also completed into potential relationships between *Lemna* toxicity and regulated chemical parameters measured for compliance purposes.

**MP062 Protection of Pollinating Species from the Effects of Pesticides: From Concerns to Protection Goals and Decision Making Criteria** A.

Alix, Ministry of Agriculture, DGAI – SDQPV – BRMMI, Ministry of Agriculture, DGAI – SPRSPP – SDQPV – BRMMI; M. Miles, Dow Agrosciences, Field Effects and Exposure. A global SETAC Pellston workshop was organized in January in Pensacola (FL) which aimed at bringing together the best available science regarding exposure and effects assessment methods for honey bees (*Apis mellifera*) and non-*Apis* bees. A review of existing regulatory texts, testing methodologies, risk assessments, scientific papers and monitoring data highlighted an important diversity in scientific knowledge. This indicated a need to identify scientific information and testing methodologies that are relevant for risk assessment and decision making purposes for pollinators which should be related to clearly defined protection goals. Ideally protection goals should be defined to be i) relevant with regard to the decision to be made; ii) justified in relation to up-to-date scientific knowledge; iii) consistent with regard to the level of protection; iv) harmonized within a specific legal framework; v) proportionate to the problem and vi) practicable i.e., reasonably easy to reach. With respect to these criteria the workshop sought to define protection goals for pollinating species which could be linked to assessment and measurement endpoints from studies. E.g., pollination may be easily recognized as a relevant protection goal for decision making that easily matches with most of the criteria. The protection of pollination implies the presence in the crop of pollinating species in sufficient abundance over the flowering period, as assessment endpoints. In the case of honey bees, it implies to protect colony survival and strength and for non-*Apis* species, to protect population size and persistence over time. Pollination is also identified as an ecosystem service and the need to protection both ecosystem services and the biodiversity of agricultural landscapes was recognized. Possible protection goals were established that can be considered further within countries and competent authorities based on their legal context. The important achievement of this workshop is to provide a basis to construct and define protection goals on a common knowledge and reasoning. This, for pollinators as for other areas of protection, will facilitate communication between countries and competent authorities, enable work sharing and achieve protection goals at a global scale.

**MP063 Generic Problem Formulation for Assessing Pesticide Risks to Pollinators** D.L. Fischer, Bayer CropScience, Global Development North America, Ecotoxicology, Bayer CropScience, Dept of Ecotoxicology; P.

Delorme, Pest Management Regulatory Agency, Health Canada. As part of a Pellston Workshop, generic problem formulations for assessing risk to honey bees were prepared for two pesticide application scenarios: (1) foliar spray application of a non-systemic product and (2) application of a systemic product to seeds or soil. While not covering all scenarios of interest (for example, non-*Apis* species were not covered), these documents aided workshop participants in understanding the risk assessment process and the need to clearly identify up front the objectives and scope of the assessment. The PF also helped clarify the linkage between identified management goals, assessment endpoints, and measures of exposure and effects for both honey bee colonies and individual bees. Assessment and measurement endpoints,

conceptual models, risk hypotheses and analysis plans for each scenario will be presented.

**MP064 Assessing the Comparative Risk of Plant Protection Products to Honey Bees, Non-target Arthropods and Non-*Apis* Bees** M. Miles, Dow Agrosciences, Field Effects and Exposure; A. Alix, Ministry of Agriculture, DGAI – SDQPV – BRMMI, Ministry of Agriculture, DGAI – SPRSPP – SDQPV – BRMMI; V.J. Kramer, Dow AgroSciences, LLC. Risk assessments are conducted for plant protection products (PPP) with respect to potential impacts on non-target species. These include pollinators such as the honey bee but also to other non-target arthropods (NTA). In common with other areas of ecotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection/conservatism. Tier I screening risk assessments are intended to rapidly exclude those substances which pose a low risk to non-target organisms and to focus resources on those for which a potential risk cannot be excluded and further studies may be undertaken to characterize the conditions and occurrence of risks. In the European Union a Hazard Quotient (HQ) approach is used to assess the risk to both honey bees non-target arthropods at Tier I. This is calculated by dividing application rate by the LR50 (Lethal Rate 50). In other regions such as North America, a contact toxicity trigger of 11 µg active substance/bee is currently employed. At the Pellston workshop it was suggested that for a risk assessment for sprayed products the honey bee could be a suitable surrogate species. However, to account for potential differences in the sensitivity between the honey bee as a test organism and other non-*Apis* bees a safety factor of 10 (for interspecies differences) was suggested. On the basis of ecotoxicological data of the European data sets for honey bees and NTA, the relative risk of PPP to NTA, honey bees and non-*Apis* bees, as depicted by HQ values is compared and where possible data on NTA pollinators (e.g., Syrphids) and non-*Apis* bees (e.g., *Bombus* sp.) are also included. At tier I using HQ trigger of 50 for honey bees, 2 for NTA, in accordance with the current EU legislation, and 5 (i.e., 50 divided by the extra factor of 10 abovementioned) for non-*Apis* bees, the NTA scheme identified more compounds and uses to move forward for further evaluation. The suggest non-*Apis* HQ trigger of 5 gave a similar pass, fail rate to the NTA scheme but was slightly confounded by a lack of determined end points. However, even taking this into consideration the most restrictive tier I assessment was that for NTAs. This poster examines the potential for a tier I risk assessment to cover NTA, honey bees, non-*Apis* bees and other arthropod pollinators as part of a tiered risk assessment scheme and compares the empirical HQ approach with methods using more specific species exposure estimates.

**MP065 Review of Higher Tier Methods for Assessment of the Risk of Pesticides to Honey Bees** M. Miles, Dow Agrosciences, Field Effects and Exposure; A. Alix, Ministry of Agriculture, DGAI – SDQPV – BRMMI, Ministry of Agriculture, DGAI – SPRSPP – SDQPV – BRMMI; V.J. Kramer, Dow AgroSciences, LLC. Risk assessment procedures have been developed for domesticated honeybees (*Apis mellifera*) potentially exposed to residues of insecticides. After initial tier I screening risk assessments further studies may be conducting for those substances for which a potential risk cannot be excluded. Higher tier studies are often performed in cages or tunnels containing colonies of honey bees which can forage on treated crops. Cage test are typically smaller in size than tunnels but allow for the testing of more treatments often with more replication than can be offered by the larger scale tunnel tests. Field tests can be conducted to establish the effects of the product under more realistic conditions but due to use of free flying colonies there is less control than in the tunnel and cage studies. Finally, post-registration monitoring can offer additional information on specified uses of the product under commercial conditions and can be used to give feedback on the outcome of the risk assessment and the effectiveness of any risk management practices put in place to protect bees. This paper will review the various higher tier methods with respect to their ability to accurately predict the potential effects of pesticides to honey bees and how they can be employed in a robust risk assessment. The range of parameters investigated typically include; mortality, foraging, behavior, brood and colony development. The ability of higher tier studies to predict potential adverse effects will be evaluated and how these parameters can be employed in a risk assessment with clear decision making linked to honey bee and pollinator protection goals.

**MP067 Movement of Soil-Applied Systemic Insecticides into the Pollen and Nectar of Squash: Are Concentrations High Enough to Impact**

**Pollinators?** B. Eitzer, Connecticut Agricultural Experiment Station, Analytical Chemistry Dept; K. Stoner, Connecticut Agricultural Experiment Station. There has been much recent interest in the threat to honey bees and other beneficial insects posed by the use of pesticides. In particular, there is a concern that systemic insecticides can translocate from the soil into pollen and nectar of plants, where they are then liable to be ingested by pollinators. We report here on the movement of two such systemic neonicotinoid insecticides, imidacloprid and thiamethoxam, into the pollen and nectar of flowers of squash (*Cucurbita pepo* cvs. "Multipik," "Sunray" and "Bush Delicata"). The insecticide treatments were within labeled rates for these compounds, either after soil application before seeding, or, by a single application through drip irrigation after transplant. Pollen and nectar were collected during the subsequent growing season. Pollen and nectar samples were analyzed utilizing a QuEChERS extraction protocol and liquid chromatography mass spectrometric analysis. With the mass spectrometer operated in a positive ESI-MS/MS mode. The concentrations found in nectar (mean  $\pm$  s.d. :  $10 \pm 3$  ppb for imidacloprid;  $11 \pm 6$  ppb for thiamethoxam and pollen ( $14 \pm 8$  ppb for imidacloprid;  $12 \pm 9$  ppb for thiamethoxam) are at the high end of the previously documented ranges for these compounds in these matrixes. These concentrations fall into the range being investigated for sublethal effects on honey bees and other beneficial insects and are therefore of potential concern.

**MP068 Field Investigation of the Safety to Honey Bees of Applications of Movento® Insecticide to Citrus During Bloom** R.E. Rogers, Bayer CropScience, LP, Ecotoxicology; D.L. Fischer, Bayer CropScience, Global Development North America, Ecotoxicology, Bayer CropScience, Dept of Ecotoxicology; G. Williams, Dalhousie Univ, Dept of Biology. The Asian citrus psyllid (*Diaphorina citri*) is probably the single greatest insect threat to the global citrus industry. *D. citri* transmits the bacteria responsible for Huanglongbing (HLB), or citrus greening disease, which causes premature defoliation, poor fruit production, and eventual death of trees. Movento® (a.i., spirotetramat) is a unique two-way systemic foliar insecticide that is effective in controlling psyllid. Movento is relatively non-toxic to many beneficial arthropods, including adult honey bees, however there were questions whether it might cause toxicity to bee larvae and as a result affect bee colony survival if sprayed during bloom. To address this question, a field trial was conducted in 2009. Twelve colonies were obtained from cooperating commercial beekeepers were placed at the edge of each of two Sweet orange, *Citrus sinensis* (variety Hamlin) groves in Pasco County, FL at the start of bloom and removed after bloom finished. One grove received a single application of Movento at full label rate of 10 oz/ac (730 ml/ha) and the other received no treatments. Colonies were assessed over a 6-mos period post-application for strength and health (i.e., adult and brood populations, food stores, pests, and diseases), and pollen, honey and wax samples were collected and analyzed for presence of residues of spirotetramat and other pesticides. There were no statistically significant differences in any measurements, including colony strength and health, brood success, hive weight change, and intra-hive mortality. Colony health of both the treatment and control groups started declining in July and by the end of the study, 25% and 42 % colony mortality had occurred in the treatment and control groups respectively. At the time of study termination, many of the living colonies of both groups were in a weakened state and in the process of collapsing. All colony losses could be attributed to biotic factors including Varroa, deformed-wing virus, Nosema, queen loss, and co-infections. Residues of spirotetramat and its enol metabolite in bee-collected nectar and pollen peaked shortly after application and were < 0.04 ppm in bee-collected nectar and 0.55 ppm in bee-collected pollen. Whole blossom residues were much higher, with a peak of 3.32 ppm. Movento residues did not persist long in bee hives. No quantifiable residues were present in samples of stored honey, pollen, or wax by day 46 post application. Blossoms were sampled when the trees bloomed again in 2010 to evaluate the potential of residue carry-over from year to year. No detectable residues were present in these samples. A question was raised regarding whether the bees at the treatment site foraged extensively on the pollen from the citrus trees as opposed to pollen from other plants in adjacent areas. A follow up study quantified the proportion of citrus pollen in the diet of bees placed at the same citrus grove in 2011. Based on the results obtained, we conclude that applications of Movento® insecticide to citrus during bloom is unlikely to harm honey bees at the individual bee and brood level as well as at the colony level.

**MP069 Effects of Contaminants on the Reproductive Success of American Eels** A. Carey, Univ of Massachusetts Dartmouth, Dept of Biology; A. Roe, US Fish and Wildlife Service; K. Oliveira, W. Hable, Univ of Massachusetts Dartmouth, Dept of Biology. The recent decline in worldwide populations of Anguillid eels is thought to be caused by pollution, overfishing, climate change, and/or habitat destruction. American eels, *Anguilla rostrata*, are known to inhabit contaminated rivers for the majority of their lives until they migrate to the ocean to spawn. Due to their unique catadromous life cycle, mature adults are not accessible for study and little is known about the effects of contaminants on fertilization and larval development. Using methods similar to the maturation of *Anguilla japonica*, *A. rostrata* males and females were artificially matured with weekly hormone injections. Using these procedures, our objectives were to (1) examine the maternal transfer of environmental contaminants to the eggs, (2) examine the effects of contaminant load on the fertility of females, and (3) examine the effects of polychlorinated biphenyls (PCBs) on the reproductive success of male eels. To examine the maternal transfer of contaminants to the eggs, ovulated eggs and muscle tissue are being analyzed for PCBs, dioxins and furans, pesticides, polybrominated diphenyl ethers, and metals. Results, to date, provide important baseline data and will facilitate development of a model for predicting parental contaminant contribution to offspring. To examine the effects of contaminant load on the reproductive potential of females, time to full maturation, ovulation, and fertilization success were evaluated and will be compared to the contaminant load in eggs. To examine the effect of PCBs on male gametogenesis, fertilization, embryogenesis and early larval development, males were co-injected with 2 different concentrations (1.0 or 10 µg/fish) of PCBs. Fertilization success and embryogenesis were assessed 2-4 hours post fertilization (PF), 24 hours PF, and 48 hours PF. Preliminary results show that males injected with 10 µg of PCBs exhibited lower reproductive success and produced embryos with disrupted early embryonic development. Fertilization success was significantly lower in embryos fertilized with sperm from males injected with 10 µg PCBs compared to the controls. Our results will shed light on whether environmental contaminants are contributing to the decline of *A. rostrata*. These findings will be important for future conservation efforts and the management of the American eel, an ecologically and commercially important species.

**MP070 Relative Sensitivity of American Eel, Lake Trout, and Rainbow Trout to Induction of Cytochrome P450 Enzymes by 2,3,7,8-TCDD** G.L. Cutler, Queen's Univ, Pharmacology and Toxicology; J.D. Byer, T. Arthur, Queens Univ; M. Alaei, Environment Canada; R.S. Brown, Queen's Univ, Dept of Chemistry; P.V. Hodson, Queen's Univ, School of Environmental Studies. A catastrophic decline in the recruitment of American eel (*Anguilla rostrata*) to Lake Ontario, starting in 1984, resulted in the extirpation of the species and its commercial fishery in Lake Ontario by 2004. One hypothesis to explain this decline is the food-web accumulation of dioxins (TCDDs, TCDFs) and other dioxin-like contaminants (DLCs) to concentrations toxic to their embryos, analogous to impacts on lake trout (*Salvelinus namaycush*) in Lake Ontario. To assess whether eels would be affected to the same extent as lake trout, we compared the relative sensitivity of juvenile eels, rainbow trout (*Oncorhynchus mykiss*), and lake trout to induction of cytochrome P450 (CYP1A) enzymes, a hallmark sign of TCDD exposure and potential toxicity. The relative potency among DLCs for CYP1A induction has been used to establish or reinforce Toxic Equivalent Factors for DLCs in risk assessment. The liver CYP1A enzyme activity of juvenile eels responded more quickly to an injection of TCDD and to a greater extent than rainbow trout, which in turn, was somewhat more sensitive than lake trout. If the sensitivity to CYP1A induction is a reasonable predictor of sensitivity to toxicity, these results suggest that reproduction of Lake Ontario eels could be impaired in the same way as lake trout, i.e., by maternal transfer of embryo-toxic DLCs. Analysis of TCDD toxic equivalent quantities and CYP1A enzyme activity in eels from other locations in eastern Canada indicated that Lake Ontario eels were most exposed to DLCs. Relatively high CYP1A activities of juvenile yellow eels suggest that contamination begins even before they reach Lake Ontario.

**MP071 Morpholino Gene Knockdown in Adult *Fundulus heteroclitus*: Role of Serum Glucocorticoid Kinase 1 in Saltwater Acclimation** E. Notch, Dartmouth Medical School, Dept of Microbiology and Immunology, Mount Desert Island Biological Laboratory, Dartmouth Medical School, Dept of Physiology; J.R. Shaw, Indiana Univ, The School of Public and Environmental Affairs; B. Coutermarsh, Dartmouth Medical School,

Dept of Microbiology and Immunology; M. Dzioba, Mount Desert Island Biological Laboratory; B. Stanton, Dartmouth Medical School, Dept of Microbiology and Immunology. The Atlantic killifish (*Fundulus heteroclitus*) is a euryhaline teleost used extensively for studies related to salt balance due to their ability to acclimate to rapid and considerable alterations in salinity. Acclimation from fresh to salt water requires remodeling of the gill from NaCl absorption to secretion to maintain osmotic balance. Chloride secretion is a multi-step process, requiring uptake across the basolateral membrane by the Na<sup>+</sup>K<sup>+</sup>2Cl<sup>-</sup> co-transporter, prior to secretion across the apical membrane via the cystic fibrosis transmembrane conductance regulator (CFTR) Cl<sup>-</sup> channel. Previous research has shown that rapid acclimation of killifish from fresh to salt water (SW) is mediated by trafficking of CFTR from intracellular vesicles to the plasma membrane in the gill within the first hour in SW, without changing overall CFTR protein levels. Acute transition to SW is also marked by an increase in both mRNA and protein levels of serum glucocorticoid kinase 1 (SGK1) within 15 minutes of transfer. Although the rise in SGK1 in gill after SW transfer precedes the increase in plasma membrane CFTR, the direct role of SGK1 in elevated membrane CFTR has not been established. To test the hypothesis that SGK1 mediates the increase in plasma membrane CFTR, we designed two different SGK1 vivo-morpholinos to knock down SGK1, developed a morpholino knock down technique for adult killifish and an approach to measure plasma membrane CFTR in the gill. Although morpholinos have been extensively used in zebrafish and other model organisms, they have not been utilized in adult killifish. Injection (IP) of adult killifish with a splice blocking SGK1 vivo-morpholino resulted in a significant, 66% reduction ( $p < 0.05$ ) in SGK1 mRNA in the gill after transition from fresh to SW for 1 hour. Injected killifish also exhibited a 64% and 53% reduction SGK1 protein levels in the gill for the translational blocking morpholino ( $p < 0.05$ ) and splice blocking morpholino ( $p < 0.05$ ) respectively. Knock down of SGK1 also reduced, by 44% ( $p < 0.05$ ), CFTR in the plasma membrane of the gills of SW fish. Thus, the increase in SGK1 is required for the trafficking of CFTR to the plasma membrane of the gill in killifish transferred from fresh to SW. This is the first report of the successful use of morpholinos in adult killifish, which provides a valuable genetic tool for this environmentally relevant model organism. (Supported by a grant from the NIEHS P42 ES007373).

**MP072 The Effects of Ethinylestradiol (EE2) on the Reproductive Axis in Female Mummichog (*Fundulus heteroclitus*)** M.A. Doyle, Univ of New Brunswick; C.J. Martyniuk, Univ of New Brunswick, Dept of Biology/Canadian Rivers Institute, Univ of New Brunswick, Biology; T. Bosker, Univ of New Brunswick, Canadian Rivers Institute; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology. Studies using freshwater fish models have shown that exposure to low concentrations of 17  $\alpha$ -ethinylestradiol (EE<sub>2</sub>) will result in a reduction or complete shutdown of egg production. Unlike other teleost fishes, the mummichog (*Fundulus heteroclitus*), an important estuarine fish model, is able to continue to produce and fertilize eggs at >3000 ng/L EE<sub>2</sub>. Previous studies have shown that mummichog respond to lower concentrations of EE<sub>2</sub> at the physiological (steroid production) and molecular (vitellogenin induction) levels. The objective of this study was to characterize genomic responses to gain insight into how mummichog are able to produce eggs normally at high concentrations of EE<sub>2</sub>. Adult mummichog were exposed under static renewal conditions for 14 d to a solvent control, 50 or 250 ng/L EE<sub>2</sub>. A suite of endpoints were assessed for females at different levels of biological organization, including whole organism (relative gonad size, liver size, condition factor, reproductive stage), physiological (steroid hormones) and molecular (real-time PCR in both gonadal and liver tissue). The focus for targeted gene expression responses were lipid mobilization and transport (e.g., apolipoprotein E, lipoprotein Lipase, hepatic lipase, apolipoprotein C-II) in the liver and steroidogenesis (e.g., Hsd11b2) in the ovary, which are two important estrogen-regulated pathways involved in egg production. For higher level endpoints, fish exposed to 250 ng/L EE<sub>2</sub> had a slightly higher condition factor than control fish. However, there were no significant differences in GSI, LSI, or differences in histology based upon the count of follicles in primary and secondary growth in each group. There was a significant induction in hepatic *vtg* mRNA (2 and 20-fold) for 50 or 250 ng/L EE<sub>2</sub> respectively. There were no significant changes in lipid-related transcripts or steroidogenic genes after EE<sub>2</sub> exposure. Based on these results, a mummichog microarray will be used to further probe the mechanisms by which mummichog maintain egg production during high exposure levels to EE<sub>2</sub>.



**MP073 Using *Fundulus heteroclitus* to Study Reproductive and Developmental Effects of Benzo[a]pyrene** C. Thornton, Univ of Mississippi, Dept of Pharmacology and Environmental Toxicology Research Program, Univ of Mississippi, Pharmacology; F. Booc, Univ of Mississippi, Pharmacology; X. Fang, Univ of Mississippi; A. Lister, Wilfrid Laurier Univ; D. MacLachy, Wilfrid Laurier Univ, Canadian Rivers Institute, Wilfrid Laurier Univ, Dept of Biology; K.L. Willett, Univ of Mississippi, Environmental Toxicology Research Program, Univ of Mississippi, Dept of Environmental Toxicol. *Fundulus heteroclitus* are exposed to polycyclic aromatic hydrocarbons (PAHs) in the wild, and thus, they are relevant organisms for studying the toxicological effects and mechanisms of PAHs. PAHs are a health concern because they are implicated in cancers and developmental deficits. We hypothesize that benzo[a]pyrene (BaP), a model PAH, can deregulate the steroid hormone hypothalamus-pituitary-gonad feedback loop adversely affecting reproductive development and physiology. Our previous work has shown that BaP downregulates aromatase (CYP19) expression. The goal of our current work is to further establish BaP-mediated toxicological endpoints including phenotypic (gonad morphology and reproductive success) and molecular consequences (steroid concentrations, vitellogenin, LH and FSH expression). A short-term reproductive bioassay was done using *Fundulus* adults exposed to waterborne concentrations of BaP (0, 1 or 10 µg/L) for 28 days. Males and females were kept separate days 0-14. The sexes were combined for days 14-28 and egg production and reproductive success was measured. BaP exposure did not alter male or female gonad somatic index (GSI) or liver somatic index (LSI) significantly. Similarly, sperm counts, egg production and fertilization success ( $n = 6$  tanks per treatment; 3 fish of each sex per tank) were not altered by exposure. Oocytes were staged based on diameter of each oocyte, but oocyte size distribution was not significantly different between treatments ( $n=5$  fish/treatment). Vitellogenin was significantly increased in 10 µg/L BaP-treated females compared to control and 1 µg/L BaP-treated females after 4 weeks exposure. Parentally exposed embryos were raised in clean water and collected for: steroid concentrations on 5 and 10 days post-fertilization (dpf) and 1 and 3 weeks post-hatch (wph); aromatase on 10 dpf and 12 wph; vasa at stage 24 (~3 dpf) and 12 wph; and histology at 1, 3 and 12 wph. In conclusion, BaP-mediated changes in aromatase expression have the potential to cause changes in the HPG axis, but reproductive success was not significantly compromised in the parental generation by up to 10 µg/L BaP exposure. Supported by NIEHS R03 ES018962.

**MP075 Comparison of Topical Treatment and Nano-injection to Investigate the Effects of PCB126 in *Fundulus heteroclitus* Embryos** C.M. Couillard, Fisheries and Oceans; C. Rigaud, Université du Québec À Rimouski; B. Legare, Fisheries and Oceans. Controlled dosing of fish embryos with lipophilic contaminants to mimic maternal transfer and investigate toxic effects is hard work. In this study, we compare the toxic responses of *Fundulus heteroclitus* embryos following topical treatment or nano-injection with 3,3',4,4',5-pentachlorobiphenyl (PCB126). A few hours after fertilisation, eggs were treated topically with 3.1 to 50 pg egg<sup>-1</sup> PCB126 in DMSO or nano-injected with 1.6 to 25 pg egg<sup>-1</sup> PCB126 in triolein. Two experiments were performed with each dosing technique. Four days post-hatching (dph), whole body EROD activity was measured in larvae. The dose-response relationships for EROD activity were compared between replicates for each dosing technique and between dosing techniques. Slopes of the dose-response relationships did not differ between dosing techniques, indicating a similar mode of action with topical or nano-injection treatments. The intercepts did not differ between replicate experiments for each dosing technique indicating that both these modes of treatments are reliable and accurate methods to expose *Fundulus* embryos to PCB126. As expected, intercepts were lower for the topical treatment than for nano-injection since only a fraction of the load of PCB126 (14(9-23) %) deposited on the surface of the eggshell reaches the internal tissues of the embryos. Both topical treatment and nano-injection caused a dose-responsive reduction in body length and in the prey capture ability. Topical treatment is less technically complex, less costly in labour and material, and less invasive than nano-injection. After validation of the internal dose, topical treatment is a valid alternative to explore biological responses and mechanisms of action of single chemicals such as PCB126. Nano-injection remains the method of choice to compare the toxicity of different chemicals, to estimate toxic equivalent factors or to assess the toxicity of chemical mixtures.

**MP076 CYP1A in South American Cyprinodontiform Fishes: New Models for Toxicological Studies** R.S. Ferreira, F.S. Guimaraes, Universidade Federal do Rio Grande, Instituto de Ciências Biológicas; A.C. Bainy, Universidade Federal de Santa Catarina, Bioquímica; J. Stegeman, Woods Hole Oceanographic Institution; J. Zanette, Universidade Federal do Rio Grande, Instituto de Ciências Biológicas. Following the example of the North American Cyprinodontiform killifish (*Fundulus heteroclitus*), South American Cyprinodontiform fishes are potential candidates to be used as model species in toxicological studies. Although poorly studied so far, those fishes possess one or more of the following motivating features: (1) Cosmopolitan and adapted to live in a wide range of stressing conditions (e.g., pollution, salinity, oxygen and temperature stresses); (2) Different from killifish, most are ovoviviparous, being interesting models to understand mother-embryo toxicological interaction; (3) A huge diversity of species exists, and possibly a diversity of adaptive mechanisms/sensitivity to environmental stress; (4) Some species are rare, thus possibly endangered. We sampled three Cyprinodontiform species that occur around Rio Grande (RS, Brazil) and sought cytochrome P450 family 1 subfamily A (CYP1A) sequences, using degenerate primers, Polymerase Chain Reaction (PCR), cloning and sequencing. Partial sequences obtained (540-655 nucleotides) for *Phallosceros caudimaculatus* (Poeciliidae), *Poecilia vivipara* (Poeciliidae), and *Jenynsia multidentata* (Anablepidae), respectively, shared 89%, 89% and 90% identity in the predicted amino acid sequence with the corresponding region of *F. heteroclitus* CYP1A. The three fish species were exposed for 8 or 24-hrs to 1 µM β-naphthoflavone (BNF), a potent AHR agonist. *P. caudimaculatus* showed a strong CYP1A induction (32-fold) in gill ( $0.88 \pm 0.26$  in BNF comparing to  $0.03 \pm 0.02$  in control;  $p < 0.0005$ ), and 49-fold in liver ( $10.50 \pm 6.29$  in BNF compared to  $0.21 \pm 0.13$  in control;  $p < 0.0005$ ), after 24-hrs, as quantified by RT-qPCR. This is the first study to identify a *P. caudimaculatus* CYP1A sequence, and to show that this transcript could be a biomarker of exposure to AHR agonists in this species. This would be useful for the environmental monitoring studies, as *P. caudimaculatus* is widely distributed in the South of Brazil. Analyses of CYP1A and responses of the other two species are underway. Our objective is to determine whether or not distinct intra- and inter-specific differences in induction of CYP1A occur and whether there may be acquired resistance or increased susceptibility to high-level contaminant exposure in South American Cyprinodontiform fishes, as occurs in *Fundulus* in N. America. (Support: INCT-TA, NIH P42ES007381).

**MP077 Bisphenol-A Effects in CYP1A and CYP19A2 Gene Expression in the South American Fish *Poecilia vivipara*** J. Zanette, S.M. Abril, Universidade Federal do Rio Grande, Instituto de Ciências Biológicas; C.R. Silveira, L.F. Marins, P.E. Martinez, Universidade Federal do Rio Grande. The guppy *Poecilia vivipara* (Poeciliidae, Cyprinodontiform) is widely distributed in estuarine regions of the South American Atlantic coast serving as a potential model specie candidate to be used in toxicological studies. The objective of this study was to identify the brain aromatase sequence in *P. vivipara*, recognized in fish species as the cytochrome P450 of the family 19 subfamily A isoform 2 (CYP19A2), and to evaluate the effects of Bisphenol-A (BPA) exposure (2, 10 and 100 mg.L<sup>-1</sup>) in the CYP19A2 transcriptional regulation in juvenile fish (0.6-0.9cm). The cytochrome P450 1A (CYP1A) gene expression was also evaluated. CYP19A2 were identified using degenerate primers, PCR, cloning and sequencing, allowing the identification and preliminary annotation of a 159 nucleotide fragment sharing 85 % in the predicted amino acid sequence, with the corresponding region of *Fundulus heteroclitus* CYP19A2. The identified region corresponded to the CYP19A2 SRS4 and helix-I region. Gene expression levels were evaluated by RT-qPCR using *ACT* as normalizing gene. The CYP19A2 and CYP1A did not showed differences comparing the exposed groups with the control group ( $p > 0.05$ ). CYP19A2 is supposed to be up-regulated via estrogen receptor (ER) in fish's brain and gonad after exposure to the endocrine disruptor BPA. The unexpected absence of CYP19A2 alteration in this study could indicate that *P. vivipara* CYP19A2 regulation differs from the previously studied species. An experiment with adult exposure to BPA and organ-specific RT-qPCR analysis is underway to investigate this hypothesis. A strong positive correlation between gene expression level and BPA concentration was observed for CYP1A ( $p < 0.05$ ), but not CYP19A2, indicating that BPA could be involved in the CYP1A up-regulation. Regulatory mechanisms such cross-talking between AHR and ER receptors could be involved. Interestingly, it has been shown recently in zebrafish, that different to mammals, CYP1A and CYP1C1 are probably the major enzymes involved in the estrogen

metabolism in fish. Experiments investigating the possible involvement of CYP1A regulation in the BPA endocrine disruptor effects are underway. This is the first study identifying a partial *CYP19A2* sequence in *P. vivipara* and investigating the gene expression regulation by the endocrine disruptor BPA in this species (INCT-TA).

**MP078 Developing the Mosquitofish (*Gambusia* spp.) as a Model Organism for Evaluating the Effects of Endocrine Disruptors in the Environment**

**E.K. Anderson**, Univ of Florida, Physiological Sciences; D.S. Barber, Univ of Florida, Dept of Physiological Sciences; Y. Ogino, Okazaki Institute for Integrative Bioscience; T. Bargar, US Fish & Wildlife Service; T. Iguchi, Okazaki Institute for Integrative Bioscience; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. Mosquitofish (*Gambusia* spp.) have the potential to become a useful bioindicator organism for endocrine disruptors. Male mosquitofish have a secondary sexual characteristic—an extension of the 3<sup>rd</sup>–5<sup>th</sup> anal fin rays used for internal fertilization—that is inducible in females and fry after androgen exposure. Female Eastern mosquitofish (*G. holbrooki*) with masculinized anal fins are still present at a paper mill-impacted site, over 30 years after their first discovery. Issues with using mosquitofish as a bioindicator include the lack of knowledge of how abnormal anal fin growth corresponds to higher-level effects such as reproduction. In addition, the molecular mechanisms that regulate anal fin growth are still unknown. To gain insight into these endpoints, three specific aims were developed: 1) Create a custom mosquitofish microarray, 2) Quantify the hepatic gene expression of vitellogenin (vtg) after androgen exposure, and 3) Observe changes after androgen exposure in the expression of three genes that regulate anal fin growth: sonic hedgehog (Shh), muscle segment homeobox C (MsxC), and fibroblast growth factor receptor 1 (Fgfr1). For aim 1, a *G. holbrooki* cDNA library was developed using the MINT-universal kit (Evrogen). Abundant transcripts were reduced using TRIMMER (Evrogen) to create a normalized cDNA library that was sequenced using the Illumina Genome Analyzer IIx. Annotation produced 20,879 contigs < 180 bp long with an E-score < 10-4. Additional *G. holbrooki* 454 pyrosequencing data will be used to improve annotation and enable probe design. For aim 2, female *G. holbrooki* were exposed to a vehicle control or one of three doses of the androgen 17beta-trenbolone for 3, 7, 14, or 21 days. Body mass, body length, and anal fin length were assessed, and livers were removed for quantitative polymerase chain reaction (qPCR) analysis. Results demonstrate that a high dose of 17beta-trenbolone (10 µg/L) significantly reduced hepatic vtg gene expression while a lower dose (1 µg/L) could induce anal fin growth (one-way ANOVA,  $p < 0.05$ ). For aim 3, the expression of 3 genes with a known role in normal anal fin growth were evaluated in female mosquitofish after exposure to 17beta-trenbolone using in situ hybridization and qPCR to evaluate the pathways involved in androgen-induced anal fin growth. These data will provide critical information on how the mosquitofish can continue to be developed as a bioindicator for endocrine disruptors.

**MP079 Quantification of Genetic Changes in the Brown Bullhead**

**(*Ameiurus nebulosus*) Exposed to Environmental Toxins** **R. Williams**, A. Hubberstey, Univ of Windsor, Dept of Biology. Aquatic environments are under a constant influx of pollutants which directly affect organisms living in these bodies of water. One species found in polluted environments is the brown bullhead (*Ameiurus nebulosus*). The bullhead is abundant throughout North America and is found in both clean and contaminated bodies of water in Southern Ontario, Canada. These philopatric, benthic fish undergo prolonged contact with sediment during feeding and other activities (where contaminants in water prominently accumulate), resulting in high levels of exposure. Because of their ability to withstand high levels of contaminant stress, these fish have been used as bio-indicators of genotoxic and contaminant stress to measure pollution induced effects on their population. The ability of bullhead to tolerate toxins has led us to propose that there may be an adaptive response occurring; allowing these fish to survive and reproduce in such areas. This response may either be a physiological acclimation or an evolutionary genetic response driven by natural selection. In order to measure changes occurring in fish and classify the type of adaptation occurring, individual genetic and molecular changes must be monitored to determine what mechanism(s) these fish are using to survive. A number of genetic markers were characterized for gene expression analysis from bullhead exposed to toxic compounds. One gene showing a marked change was p53. To determine whether the effects on p53 expression were observed in natural bullhead populations, fish caught from clean and contaminated sites

in Southern Ontario were examined for their endogenous p53 protein levels. Definitive differences were seen between levels of p53 in fish from clean and contaminated sites. Fish caught from clean sites showed lower p53 protein levels than fish from contaminated sites. Bullhead from clean and contaminated sites were also fed benzo[a]pyrene (a common PAH) treated food. Fish from clean sites increased their levels of p53 upon exposure to BaP whereas fish from contaminated sites showed decreased levels of p53 although their endogenous p53 levels were higher. This suggests an underlying differential adaptive mechanism of the bullhead living in either clean or contaminated sites. Whether this response is genetic or physiological is currently being examined. Further studies, including measuring gene expression in F1 raised offspring whose parents originated from clean and contaminated sites will also be monitored.

**MP080 Biomarkers of Pollution in the Mozambique Tilapia, *Oreochromis mossambicus*, Cichlidae**

**J. van Vuren**, Centre for Aquatic Research, Univ of Johannesburg, Zoology, Univ of Johannesburg, Dept of Zoology, Auckland Park Kingsway Campus; V. Wepener, Centre for Aquatic Research, Univ of Johannesburg, Zoology; K. Bremner, Univ of Johannesburg, Zoology; R. Basson, Univ of Johannesburg. The potential adverse effects of different contaminants present in the aquatic environment through industrial, agricultural and urban activities have to be determined to predict maximum tolerable levels for the organisms present. Information on the survival of organisms is useful in water quality management. Carefully selected biomarkers are recognised indicators of the levels of pollution that could compromise the health of aquatic organisms. Effects of contaminants on fish physiology and organ function in specific impacted areas were determined with laboratory exposure studies. These toxicity tests were specifically done to determine the molecular effects of selected chemicals and metals present in the study areas. The results obtained from the exposure experiments were also used to supplement the findings obtained during field surveys. The findings can be of use in water quality management where the prediction of environmental effects of toxicants, comparison of toxicity levels and regulation of effluent are important for the survival of organisms. The Mozambique Tilapia, *Oreochromis mossambicus*, an indigenous fish species, was used as an experimental organism in recently completed studies. This species complies with all the requirements for an experimental organism in studies of this nature. Biomarkers were carefully selected to provide specific information on physiological and biochemical changes in the liver, gills and gonads of exposed organisms. Toxic effects of the selected chemicals and metals were determined by using two groups of biomarkers. Metallothioneins, acetylcholine esterase, cytochrome P450 and glutathione-S-transferase are biomarkers of exposure and grouped together. The other group contained biomarkers of effect e.g., cellular energy allocation (indicator of energy reserves – proteins, carbohydrates, lipids; energy expenditure – electron transport system activity), oxidative stress (superoxidase dismutase, catalase, malondialdehyde, protein carbonyls, lipid peroxidase, glutathione-peroxidase), DNA damage (DNA strand length method), Metabolomics (1-D NMR), lactate dehydrogenase, alkaline phosphatase and heat shock proteins. Standard techniques were employed. Results are discussed in view of the importance of the findings to assist in the management of industrial, agricultural and urban effluent. The validity of biomarker responses in toxicity testing as a component of water quality monitoring programmes is considered.

**MP081 Addressing the Effects of Wastewater Treatment Work Effluents on the Sustainability of Populations of Fish (Roach, *Rutilus rutilus*) in English Rivers**

**P.B. Hamilton**, Univ of Exeter, Biosciences; C.A. Harris, E. Nicol, S. Jobling, T.J. Runnalls, J.P. Sumpter, Brunel Univ, Institute for the Environment; V. Vinciotti, Brunel Univ, School of Information Systems Computing and Maths; A. Henshaw, Environment Agency; D. Hodgson, T.S. Coe, J.R. Stevens, Univ of Exeter; C.R. Tyler, Univ of Exeter, School of Biosciences. Wastewater treatment work (WWTW) effluents can make up a large proportion of the flow of lowland rivers across the world, yet their effects on the sustainability of fish populations are largely unknown. WWTW effluents and some of the endocrine disrupting chemicals (EDCs) they contain induce a range of feminised phenotypes in male fish, including the intersex condition – the presence of developing eggs in the testes of otherwise male fish. Studies on roach (*Rutilus rutilus*) living in rivers in the United Kingdom have found intersex roach at 86% of river locations. We examined the ability of intersex roach to reproduce in competitive breeding scenarios. Wild roach were taken from effluent-contaminated rivers in the United Kingdom, placed in large tanks, and were allowed to breed.

Feminization of male gonads was quantified by counting the number of oocytes present in the testes, and DNA microsatellites were used to assign parentage. The influences on reproductive performance were analysed using multivariate statistics. In the first study, fish length was the only factor found to significantly influence reproductive success. In the second study, in which more fish were moderately-severely feminised, the intersex condition significantly impaired reproductive success, reducing the average contribution for the most feminised fish by 76%. The intersex condition is therefore likely to be an important influence on reproductive performance in rivers in which moderately to severely feminized males are common. Potentially effective population sizes ( $N_e$ ) could be affected and therefore the ability of populations to adapt to environmental change. We are assessing  $N_e$  across a range of rivers which receive varying levels of WWTW effluents. However, findings to date show high effective population sizes at some heavily impacted sites.

**MP082 A Morphological and Molecular Method for Assessing the Effects of Potential Toxins on Zebrafish (*Danio rerio*)** A. Shaffer, C. Graham, M. Elrod-Erickson, R. Otter, Middle Tennessee State Univ, Biology. Toxicological studies are often designed to determine the toxicity and/or teratogenic properties of a compound, but may miss or inadequately measure more subtle effects. When developing embryos are subjected to a potential toxin, an effect on the overall rate of development is possible. To enable careful measurement of such an effect, a set of methods was developed for use with zebrafish (*Danio rerio*) embryos. The transparency of these embryos, as well as their rapid development, makes them an ideal choice as a model for vertebrate development. Time-lapse video microscopy was used to record morphological changes continuously throughout embryogenesis. Morphological "milestones" were chosen for their uniqueness in order to minimize ambiguity about the timing of their appearance. Plotting the appearance of these milestones over time yields a clear representation of developmental rate. As a second measure of rate that does not rely on morphology, reverse transcriptase polymerase chain reaction (RT-PCR) was performed on RNA extracted from embryos at specific times after fertilization to monitor the detectable onset of expression of the developmentally regulated genes *shh*, *sox2*, and *tnnt1* (as well as the control gene *ef1a*). These genes are expressed only at specific points in development and thus provide a set of molecular "milestones" that can be used to corroborate the morphological data. The utility of these methods was tested on embryos developing at either the preferred temperature of 28.5°C or at 24.5°C, a temperature known to slow development without causing abnormalities. These methods were also applied to embryos exposed to sublethal doses of silver nanoparticles, where the suggestion of developmental delay has been observed.

**MP083 Challenges in Valuing Ecosystem Services** D. MacNair, T. Tomasi, Cardno Entrix. Valuing ecosystem services requires integrating basic ecological and economic concepts. The integrated model uses ecological production functions to specify the relationship among the ecological inputs and economic utility functions to specify the value of the resulting ecological outcomes. The integrated model can be used as a decision support tool to evaluate different environmental management options, whereby the scientists specify some ecological change and the stakeholders value the output. However, the devil is in the details. To work successfully, the integrated model must clearly distinguish between the inputs and the outcomes to ensure that scientists and stakeholders are assigned the appropriate tasks. For example, the model will not be reliable if stakeholders are valuing inputs based on their own assumptions about how they affect desired outcomes. This presentation will highlight some of the empirical challenges and impacts of estimating integrated economic and ecological models. SETAC participants will be given the opportunity to participate as stakeholders or scientists in an online survey prior to the conference. The survey concerns hypothetical land management decision about desert habitat. The results of the survey will be presented at the conference and will be used to explore the potential impacts and difficulties in properly specifying ecological production functions and economic utility functions.

**MP084 A Decision Framework and Model to Assess Ecosystem Services at Three Military Installation Sites** P. Booth, Exponent, EcoSciences; S. Law, Exponent, EcoSciences, Exponent, Environmental Group; J. Ma, Exponent. We developed an analytical decision framework and model to inventory and assess the tradeoffs of ecosystem services at three military installation sites. Each of the sites has a distinctly different biological and

physical environment and is required to support different military missions or uses. Implementation of the framework starts with the compilation of relevant data and implementation of a stakeholder engagement process, to elicit specific preferences for ecological services and their relative importance (for use in preference weighting) from installation decision makers as well as other affected parties. The ecosystem services identified by stakeholders are linked by ecological production functions to specific ecological endpoints. Ecological endpoints are quantified using geospatial analysis (preferred) or from field surveys. The relative value of ecosystem services is quantified using existing, scientifically-vetted models or models developed for a site-specific endpoint. The outputs from each ecosystem service model are combined to determine the relative value of all ecosystem services at spatially-explicit locations. At each installation site, the relative value of each ecosystem service is defined and scenarios are presented to stakeholders, to quantitatively illustrate tradeoffs of ecosystem services and their effects on the military mission. An ecosystem services-based scenario building allow stakeholders to make better informed decisions to maximize the value of resource services as well as fully understand mission dependencies on ecosystem services.

**MP085 Implications of Life History and Model Choice on Bird Population Recovery Estimates after Pulsed Contaminant Exposure** C. Meyer, Arcadis US, Inc.; J. Meyer, Arcadis US, Inc., ARCADIS. Predictions of time to recovery of populations exposed to pulses of contaminants vary depending on the life history of the organism and the population model used. Population models used can range from density-independent models to various density-dependent models such as the ceiling model, Gompertz, Ricker, Beverton-Holt, or theta-logistic models. The models can be deterministic or stochastic. We evaluated how different combinations of models and bird species influence outcomes of pulsed-contamination scenarios. We restricted our evaluation to the dynamics of species at or fluctuating around carrying capacity (K), because most vertebrates that are not threatened and endangered are near K when exposed. Using available time series covering low to high densities, population growth rates near K were estimated by fitting the time series to the theta-logistic model. A species that maintains a high maximum population growth rate until near K (high theta) can recover more quickly from a contaminant pulse than a species with a low population growth rate near K. However, species with such high population growth rates can have high population fluctuations around K. If stochasticity is included in the model, such species show results opposite of expected, with a lower probability of extinction when exposed to contaminants than when unexposed, because the contaminants decrease the population size and thus dampen the fluctuations near K. In contrast, ceiling models of species with high growth rates near K produce rapid recovery without deterministic fluctuations and do not show increased stability in the presence of contaminants. The Gompertz model produces more rapid recovery if populations are depressed to lower than half of K than the Ricker model, and has unrealistically high growth rates at very low densities. Unlike the other models, the Gompertz model can not be applied to a population having one carrying capacity and then be scaled up to a different population having a higher carrying capacity. Ideally, the model that best calibrates to the observed time series and data should be selected; but if the observed time-series data are limited in how far they diverge from K, our results suggest that the model to be used to characterize risk to populations should be carefully selected.

**MP086 EPA RSLs for Fish Ingestion: Not Protective Enough?** G.I. Greenberg, Gradient Corporation. USEPA's regional screening levels (RSL) are designed to identify areas that warrant further investigation and commonly used to identify contaminants of concern (COC) for various media in risk assessments. USEPA (2010) published RSLs for residents exposed to contaminants via fish ingestion. However, these RSLs may not be conservative enough because they are based on an outdated ingestion rate of 54 g/day, from a 1977-1978 USDA Nationwide Food Consumption Survey published in 1982. This ingestion rate, recommended by USEPA (1991) as a standard default exposure parameter, is the average consumption rate derived from a 3-day study of people who consumed finfish (not dried, canned or raw). More current fish ingestion rates have been published with a wide array of rates that include the consumption of shellfish, which could alter the current USEPA fish screening levels. In addition, USEPA's screening level does not account for the higher fish ingestion rates among subpopulations (e.g., subsistence populations, native American populations, Asian communities) as well as regions (e.g., Michigan, Louisiana). Consequently, sites or COCs with fish tissue concentrations below the screening levels may be improperly



omitted from further investigations due to the outdated ingestion rate. More recent fish ingestion rates, its bases, and how it could alter USEPA's fish regional screening levels will be presented, compared and contrasted with the current standard.

**MP087 Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Community Garden Soils in New York City (NYC)** L.G. Marquez Bravo, H.M. Spliethoff, L.N. Ribaud, D. Briggs, New York State Dept of Health, Center for Environmental Health; M.B. McBride, Cornell Univ, Dept of Crop & Soil Sciences; H.A. Shayler, D.M. Lopp, Cornell Univ, Dept of Crop and Soil Sciences; V.T. Lambert, Cornell Univ, Cornell Cooperative Extension; E. Stone, Green Thumb, NYC Dept of Parks and Recreation. Urban community gardens provide many public health benefits, but little is known about the extent and distribution of soil contaminants in these gardens. As a response to gardeners' concerns about exposure to soil contaminants, we developed a community-research collaboration under NIEHS Partnerships for Environmental Public Health. With the goal of characterizing PAH concentrations, we collected soil from 20 community gardens across five boroughs in NYC that were part of a city-wide program that encouraged importation of clean soil and compost. For each garden, one sample was collected from a non-growing area and another from a vegetable-growing bed. Additional samples were collected from beds where a visual inspection identified factors that could be influential with respect to contaminant concentrations (e.g., immediate proximity to highly trafficked roads, presence of asphalt or cinders, creosote timbers for beds, proximity to deciduous trees, etc.), for a total of 69 soil samples. The median sum of concentrations of EPA's 16 priority PAHs ("EPA PAHs") in these samples was similar to that previously reported for surface soils in NYC (5.4 vs. 6.4 ppm), but the maximum concentration in this study was 6 times higher (150 vs. 24.8 ppm). Forty percent of the sampled gardens and 65% of sampled beds had all PAH concentrations below the New York State Residential Soil Cleanup Objectives (SCOs) (based on health and background) used in this study as guidance values. Concentrations of EPA PAHs were marginally significantly ( $p=0.052$ ) higher in non-growing areas (median=7.4 ppm) than in growing beds without potentially influential factors (median=3.5 ppm), possibly due to practices such as adding clean soil and compost to beds. No significant differences were found between beds with and without influential factors. Results were shared with gardeners along with healthy gardening strategies to reduce exposure to PAHs in gardens. Current and future studies will obtain soil-plant uptake rates in commonly grown vegetables in NYC to estimate human exposure to PAHs through vegetable consumption.

**MP088 Human Risk Assessment of Organochlorine Pesticides (OCPs) in Korea** S. Lee, Gwangju Institute of Science and Technology (GIST), School of Environmental Science and Engineering; D. Lee, B. Yim, Yeongsan River Environmental Research Center; S. Kim, Gwangju Institute of Science and Technology (GIST), Environmental Science and Engineering. Organochlorine pesticides (OCPs) have been banned several decades ago, but the evaluation of human risk on OCPs is necessary due to their persistence, bioaccumulation and toxicity. Concentrations of seven OCPs were determined in ambient water collected from 28 sampling sites in Yeong-San (YS) and Seom-Jin (SJ) Rivers in Korea. The potential health risk of OCPs associated with consumption of fish and drinking water was also assessed regionally with residual level of OCP in ambient water. Gas chromatography-high resolution mass spectrometry (HRMS) was used for chemical analysis. Uncertainties of risk assessment model and decision rule were resolved by Monte Carlo analysis. Risk characterization for OCPs was performed by calculating the ratio of the measured concentration and the water quality criteria for human protection in YS and RJ Rivers. The ratio (risk quotient, RQ) of hexachlorobenzene and heptachlor epoxide was below one, which indicates that no adverse effects may occur due to OCP exposure in ambient water. Even though RQ shows no adverse effect on human, the under-estimation of RQ may be induced by the calculation of average concentrations from left-censored data in HRMS analysis. Continual monitoring of OCPs in waters with more accurate analytical technique is required to obtain reliable human health risk.

**MP089 Pollution Prevention in the State of Sonora, Mexico: Toxic Chemicals in Health Care Centers** C. Alvarez-Chavez, M. Arce-Corrales, A. Gomez-Alvarez, E. Bolado-Martinez, Universidad De Sonora. In this study, pollution prevention efforts were started to identify the chemicals currently in use in health care centers of Hermosillo, Sonora, Mexico, that due

to their toxic properties must be reduced/eliminated in addition to mercury. The purchasing, consumption, and distribution inventories of a public medical center were revised for the years 2008, 2009, and 2010, obtaining a list of 667 products that contain chemical ingredients and that are used as detergents, disinfecting agents, sterilizers, antiseptics, stripper, paint, solvents, grease removers, chemicals, clinical thermometers, among others. This list was reduced to 87 products through the application of exclusion/inclusion criteria such as purchasing frequency and volume. The qualitative analysis of the composition of the products revealed that these contain ingredients that have a potential of causing adverse effects in human health and in polluting the environment such as glutaraldehyde, formaldehyde, mercury, ethylene oxide, thinner, sodium hypochlorite, among others. One of the barriers found for obtaining detailed information about the products was that the system inventory of the health center does not regularly include data about the composition of them, or facilitates the use and transference of data to other users. This is due to the Federal and State Government's policies that regulate the acquisition of products in public health institutions in which a general code and a generic description of each product are included. It is concluded that being healthcare the objective of these institutions, it is necessary to promote pollution prevention programs that reduce the hazards of the chemicals in use in this workplaces and also to include pollution prevention and occupational safety criteria in the purchasing policies of government. This will contribute to reduce the hazards for the use of hazardous chemicals substances for workers, public and environment, it will be useful as a guide and reference for this workplace and other health institutions in Mexico, and well as to promote innovation maintaining quality and efficiency of the health services without increasing costs, health hazards or environmental pollution.

**MP090 Priority Listing for the Reduction of the Use of Toxic Chemicals in Surgery Services of a Medical Center in Hermosillo, Sonora, Mexico** C. Alvarez-Chavez, E. Cruz-Mirazo, M. Arce-Corrales, Universidad De Sonora. Nowadays, the existing pollution prevention efforts at the medical centers in México have been focused on reducing and eliminating the use of mercury. Therefore, an analysis and prioritization of those chemicals that because of their harmful effects to the environment and health must be reduced or eliminated were done for chemicals in use in the surgery services of a medical center at Hermosillo. For this, the purchasing, consumption, and distribution inventories for the years 2008, 2009, and 2010 were revised, physical inspections were held, employees and suppliers were interviewed, as well as product labels were checked to identify the products currently in use in these services that contain chemical substances. The previous research threw out a list of 74 products that was reduced to 60 considering those that were purchased in two of the three years and excluding products in use in maintenance and general services. The 60 products are used as disinfectant agents, sterilizers, antiseptics, and in medical devices, etc. An analysis of their composition resulted in the identification of 27 chemicals. After this a methodology considering data available in literature about the harmful effects for health and environment was developed for prioritization purposes. The results showed that mercury, ethylene oxide and formaldehyde, hydrogen peroxide, benzalconium chloride, glutaraldehyde and iodine top the list that considers these criteria. The highlights of this list, besides mercury, are formaldehyde and ethylene oxide because of their potential to cause cancer and other irritating effects. These results show the need to consider other chemicals besides mercury in pollution prevention programs and policies in the health care sector in Mexico. A qualitative risk analysis to the employers during their activities in the medical center while using these products will contribute to refine this priority listing and will serve as a guide for the efforts leading to occupational health and pollution prevention in this workplace. This can also serve as reference for other health services in the region, for the elaboration or modification of pollution prevention policies in the country and to promote innovation in this field.

**MP091 Ecotoxicology and Hematological Changes in Tadpoles of *Lithobates catesbeianus* Exposed to Agrochemicals Mixture in Rice Crops** T.C. Paiva, F.M. Franca, Univ of São Paulo, Dept of Biotechnology; A.S. Marantonio, Polo APTA Vale do Paraiba; P.C. Teixeira, Univ of São Paulo, Aquaculture Center; M. Hipolito, Polo APTA Vale do Paraiba, Biological Institute; F.T. Silva, Univ of São Paulo, Dept of Biotechnology; C.M. Ferreira, Polo APTA Vale do Paraiba, Fisheries Institute. In Brazil, the rice crops has a great economical and social importance but make heavy use of agrochemicals, specially herbicides and insecticides. The main aim of this

study was to evaluate the potential toxicity to tadpoles of *L. catesbeianus* in rice crop irrigated water that receive high loads of pesticides and to determine hematological parameters. The property chosen to realize the tests is a commercial rice crop farm localized in Tremembé city, São Paulo state, Brazil. This property make use of pesticides commonly applied in the rice crops similar to others properties in the region. After 25 days of planting pre-germinated seeds, previously treated with Fipronil, the court was drained for the application of the pesticides: Penoxsulam 240g/L (150ml/ha) + vegetable oil (1L/ha), Bentazom 600g/L (2L/ha) and Permethrin 250g/L (100mL/ha). After 3 days of application, the court was again flooded and the tadpoles were exposed. And after five days about the beginning of the exposition was applied Carbofuran (500g/ha) with the fertilizer. Screened cages measuring 0.8 x 0.8 x 0.4m were fixed inside the rice crops, allowing the tadpoles to access the sediment. In each group it was used 150 tadpoles in the 31 Gosner stage (1960) with medium weight about 7.08 ± 0.49g. Before the beginning of the tests and in the experimental days 3, 7, 10, 14 and 21 it was take 6 animals/group. These animals were anesthetized to blood collection to evaluate hematological parameters: hematocrit (Ht), hemoglobine tax (Hb), counting of erythrocyte's number, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). After 21 days of exposure, it was verified the survival tax and medium weight was 100% and 6.33 ± 0.31g in the control group; 92.7% and 5.72 ± 0.28g in the exposure group. The medium values of Ht, Er, MCV and MCHC don't show significant differences: (Ht) 19.37 ± 2.29%, (Er) 21.30 ± 4.74 x 10<sup>4</sup>/mm<sup>3</sup>, (MCV) 1053.19 ± 247.15 fL and (MCHC) 19.41 ± 3.54%. Hb shows significant difference only in the 14th day, medium values of 4.39 ± 0.70g/100mL to control group and 2.74 ± 0.62g/100mL to exposure group. During the others analyzed days the Hb was kept in normal values of 3.56 ± 0.37g/100mL. The tadpoles of *L. catesbeianus* show resistance to agrochemicals used in rice crops observing the small mortality. This mortality was concentrated during the Carbofuran exposure, 6 days without change of water.

**MP092 Distributions of Mercury in Fish of the Paraná River, Brazil** B.W. Matherne, Univ of North Texas, Dept of Biological Sciences; P.M. Holt, M.M. Chumchal, Texas Christian Univ, Dept of Biology; A.P. Roberts, D.J. Hoeinghaus, Univ of North Texas, Dept of Biological Sciences; A.A. Agostinho, L.C. Gomez, Universidade Estadual de Maringá, Núcleo de Pesquisas em Limnologia. Methylmercury (MeHg) is a chemical contaminant found at measurable concentrations throughout the earth's waterways. MeHg is known to bioaccumulate within the tissues of aquatic organisms and biomagnify through food webs. MeHg contamination has been shown to affect fish survival, reproduction, and behavior as well as pose risks to human health. Biological characteristics such as fish age, size, and growth rate all have varying effects on MeHg bioaccumulation. This study focuses on understanding how MeHg is distributed in the complex food web of the Paraná River, Brazil, and examines relationships between MeHg and ecological parameters that may mediate MeHg bioaccumulation in this highly-connected and species-rich tropical food web. Samples were collected from several locations (e.g., reservoir, floodplain, rivers) along an approximately 500 km stretch of the river basin (216 fish representing 29 species, including individuals acquired from local artisanal fisherman and markets). Fourteen percent of the fish species examined had mean Hg concentrations above 300 µg/kg (wet weight), exceeding the level at which the USEPA recommends limiting consumption to avoid adverse human health effects. Results also demonstrate a positive correlation between standard length, trophic position, and Hg concentration indicating biomagnification in the Paraná River ecosystem. This study has direct applications to the quality of artisanal fisheries as a key ecosystem service utilized by local communities, and may identify species or ecological traits that correlate with low risk for MeHg in fisheries species.

**MP093 What Lessons? Phytotoxicity and Tropical Coastal Ecosystems – The Void** M. Lewis, USEPA, Gulf Ecology Division. Tropical plant-dominated ecosystems provide at least 21 ecological services valued as great as \$28000/h. These ecosystems, intertidal wetlands, seagrass meadows and mangrove wetlands/forests, have declined by as much as 50% worldwide during the last 20 years due in part to urbanization. This presentation evaluates the ability of the published toxicity database for metals, pesticides and petrochemicals to support the risk assessment process and the use of current regulatory effects-based criteria to protect near coastal, plant-dominated ecosystems. The phytotoxicities of contaminated sediments, tissue-accumulated chemicals, and water column contaminants will be summarized and shown

to be unknown for most near-coastal temperate and tropical plants based on reviews of almost 900 papers. Dose response tests and traditional LC50, EC50 and NOEC concentrations are uncommon. Toxic effect concentrations, sensitive species, life stages and response parameters are almost unknown for most chemicals. Likewise, the impact of chemicals accumulated in sediment and plant tissues is unknown. Consequently, there is a lack of predictive ability and scientific basis for management of these plant-dominated habitats as related to the presence of contaminants, either alone or in combination with other stressors. The lack of a sufficient database restricts effective risk assessments for chemicals originating from land-based runoff as well as those from episodic events such as large oil spills. The question remains whether current water quality criteria and sediment quality guidelines are protective for coastal emergent and submerged plants in either the temperate or tropical zones. Therefore, a proactive approach is needed to obtain information on the magnitude, temporal variability and biological significance of common shoreline contaminants to coastal plants inhabiting diverse geographical regions.

**MP094 A Multi-section Passive Sampler for Measuring Sediment Pore-water Profiles of Hydrophobic Organic Chemicals** L.J. Bao, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; S.P. Xu, E.Y. Zeng, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key of Organic Geochemistry. Measurements of hydrophobic organic chemicals (HOCs) in sediment porewater, a central component in assessing the bioavailability and mobility of HOCs in sediment, have been scarce. Here we introduce a multi-section passive sampler with low-density polyethylene (LDPE) as the sorbent phase, which is appropriate for measuring vertical concentration profiles of HOCs in sediment porewater of various depths. The multi-section passive sampler is composed of a series of identical sampling cells insulated with seclusion rings. In each section, sorption of HOCs into LDPE is diffusion-controlled through the water layer separated from the sediment by a glass fiber filtration membrane and a porous stainless steel shield. Laboratory testing indicated that the porewater concentrations of 1,1-dichloro-2,2-bis-(chlorophenyl)ethane (*p,p'*-DDD) and 1,1-dichloro-2,2-bis-(chlorophenyl)ethylene (*p,p'*-DDE) in spiked sediment obtained by the multi-section passive sampler were in the approximate range of those yielded through centrifugation, a conventional technique for sampling sediment porewater, and with an equilibrium partitioning approach. Field deployment of the multi-section passive sampler in an urbanized coastal region was able to obtain vertical concentration profiles of *p,p'*-DDD, *p,p'*-DDE, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (*p,p'*-DDT), 1-chloro-2,2-bis-(chlorophenyl)ethylene (*p,p'*-DDMU), 2,2-bis(chlorophenyl)ethylene (*p,p'*-DDNU), and 4,4'-dichlorobenzophenone (*p,p'*-DBP) in sediment porewater, which were deemed reasonable with a comparison with sediment concentration profiles of the same target analytes from the proximate location.

**MP095 Comparison of SPME Fiber and Tenax Methods to Predict the Bioavailability of Permethrin** A.D. Harwood, Southern Illinois Univ, Zoology, Southern Illinois Univ; P.M. Landrum, Southern Illinois Univ; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology. Recent studies have recognized the ability of chemical techniques to predict bioavailability more effectively than whole sediment concentrations. Two such techniques use SPME fibers and Tenax beads, to estimate chemical activity and bioaccessibility, respectively. While the majority of research using these techniques has been conducted using legacy compounds such as PCBs and PAHs, there is great potential for these methods to be utilized for highly toxic, rapidly biotransformed compounds such as pyrethroid insecticides. The widespread contamination and environmental risk of pyrethroids in sediments is well documented. Therefore, an accurate technique to evaluate the bioavailability of these compounds would improve environmental assessments. The objective was to compare the two techniques in their ability to predict the bioavailability of permethrin to two benthic invertebrates (*Lumbriculus variegatus* and *Hexagenia* sp.). In addition, variations of the application of the two techniques were explored. For SPME fibers, fiber co-exposure with the animals during the 28 day bioaccumulation bioassay was compared to fibers exposed separately on a shaker table. Previous studies have determined that a single Tenax extraction time of 6 or 24 hours was effective to predict bioavailability, so the relative ability of these two time periods to predict bioavailability was compared. There was no significant difference between SPME fiber concentrations between species or between the shaker table and co-exposure. This was true



among all concentrations used. There was a correlation between SPME fiber concentrations and both 6 and 24 hour Tenax concentrations. The SPME fiber concentrations, 6 h, and 24 h Tenax extractable concentrations correlated with both the total permethrin (parent plus metabolites) as well the parent permethrin concentration in the tissues of both species. When tissue concentrations were normalized for biotransformation and percent lipid both species tissue concentrations could be predicted with one relationship. This demonstrates the potential value of these methods to predict bioavailability for a range of compounds and multiple species.

**MP096 Utilizing a Thin-Film SPE Methodology to Assess the Bioavailability of DDT and Dieldrin Residues in Soils** N. Andrade, Univ of Maryland, Civil and Environmental Engineering; L.L. McConnell, USDA-ARS, Environmental Management and Biprodukt Utilization Laboratory, US Dept of Agriculture-ARS, Environmental Quality Laboratory; A. Torrents, Univ of Maryland, Civil and Environmental Eng.; M. Ramirez, District of Columbia Water and Sewer Authority; C.J. Hapeman, USDA-ARS. While DDT and dieldrin have been banned in most countries, heavily contaminated agricultural sites exist in many areas. A historical orchard that received routine DDT and dieldrin applications more than 40 years ago is being assessed for potential in situ remediation with organic carbon amendments. The remediation goal is to reduce bioavailability of pollutants to earthworms to reach standards set by USEPA. Testing residue bioavailability using earthworm bioassays is time-consuming and complex; differences in commercially-available species and experimental design may cause variability in results. In order to rapidly estimate bioavailability of these organic pollutants to earthworms and to assess remediation options, a thin-film solid-phase extraction assay has been tested. A recent study has shown that the thin-film polymer can mimic biological membranes and concentrations in the polymer were strongly correlated to residue uptake by mussels in sediment. In this experiment, DDT- and dieldrin-contaminated soil from the orchard site and freshly-spiked control soil were analyzed using this thin-film methodology, and results were compared to concentrations found in native earthworms. Preliminary results show that aged soil 4,4'-DDT reached equilibrium with the film in 10 days, while its metabolite, 4,4-DDE, reached equilibrium only after 110 days and dieldrin in 64 days. We expect that equilibrium times will be shorter for freshly spiked soil. The orchard soil was mixed with 5 different organic carbon amendments, and the change in bioavailability of the residues relative to the unamended soil was determined. Amendments included biochar, composted biosolids, limed biosolids, and two manure composts. This approach may provide a useful, rapid and easy assessment of bioavailability of organic pollutants in aged contaminated soils.

**MP097 Comparative Evaluation of Methods for Estimating Bio(availability) of Organic Contaminants in Soil** B.M. Adedigba, Univ of Lancaster, Lancaster Environment Centre. There is interest in developing a method for estimating bioavailability of hydrophobic organic contaminants (HOCs) in soil. Existing methodologies are inadequate due to limitations imposed by physical and chemical characteristics of contaminants. XAD is a group of hydrophobic resin with high affinity for organic compound in aqueous phase. It has been used to pre-concentrate trace organic compounds in solution thus facilitating their detection by analytical instruments. The aim of this study was to optimize an extraction which relies upon the desorption of organic contaminants from soil in the presence of XAD; this desorption technique may be used to estimate the bioaccessibility of HOCs in soil. The applicability of XAD technique to estimate bioaccessibility of phenanthrene (model PAH) across 5 concentrations (0, 0.01, 0.1, 1.0 and 10 mg/kg of soil) was measured. Bioaccessibility was measured in terms of biodegradation of  $^{14}\text{C}$ -phenanthrene by a culture of *Pseudomonas* sp. The success or otherwise of XAD was assessed by comparing the data with those from the well reported cyclodextrin extraction. Four sequential 22 hourly extractions were required for XAD-2 to accurately estimate biodegradation across all concentrations. In contrast, a single cyclodextrin extraction produces similar results within 12 hours. Linear correlations between cumulative XAD-2 extracted and cyclodextrin extracted data revealed a very good relationship (best fit of  $r^2 = 0.896$ , slope 1.031). On the contrary, the residual concentrations predicted by XAD-2 underestimate cyclodextrin and biodegradation endpoints by >30% and >33%, respectively. While these data suggest that XAD-2 is a promising method for estimating HOC accessibility to microflora, cyclodextrin extraction remains the most rapid technique for predicting bioaccessibility of HOCs. In view of

the limitation of cyclodextrin to predict highly persistent compounds, it is necessary to further optimise XAD adsorption for better performance

**MP098 Relationships Between Petroleum Hydrocarbon Concentrations and the Results of Toxicity Tests with Terrestrial Species** G.L. Stephenson, Stantec Consulting Ltd., Environmental Management, Stantec Consulting Ltd., Environmental Remediation Services; W. Ma, Stantec Consulting Ltd.; S. Siciliano, Univ of Saskatchewan; B. Zajdlík, Zajdlík & Associates Inc. Two Saskatchewan (Canada) soils amended with commercially-acquired diesel at concentrations ranging from 100 to 30,000 mg/kg soil dry weight were artificially aged and weathered by mechanical mixing twice weekly for three weeks prior to use in toxicity tests with plants and soil invertebrates. At the beginning of the toxicity tests, soil subsamples were extracted with cyclodextrin, a solvent mixture of hexane and acetone (1:1 v:v), and an enzyme mixture which simulated the gastrointestinal fluids of earthworms (SEG extraction). The potential petroleum hydrocarbon exposure concentrations from these extraction methods were measured using GC-FID. Biological effects (survival, seedling emergence, growth or reproduction) of the PHC-contaminated soil were assessed by conducting toxicity tests with two species each of plants (*Elymus lanceolatus* – Northern Wheatgrass; *Hordeum vulgare*– Barley) and invertebrates (earthworm – *Eisenia andrei*; springtail – *Folsomia candida*). The multi-concentration exposure series enabled the estimation of IC50s and IC25s for the various biological endpoints. Multiple regression procedures and the application of data reduction methodologies were used to determine the relationships between the effect data and the measured potential exposure concentrations as determined by the three different extraction methods predicted (correlation) effects. The influence of soil characteristics was also taken into consideration.

**MP099 Influence of Molecular Size on Chemical and Biological Availability of Sediment – Associated Contaminants** H. Li, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences; J. You, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry. Besides the widely investigated factor of hydrophobicity, molecular size could affect bioavailability of hydrophobic organic contaminants in sediment as well. To evaluate the influence of molecular size on bioavailability, four pairs of contaminants, including a polychlorobiphenyl (PCB) and a pyrethroid insecticide having similar hydrophobicity but different molecular weight in each pair, were selected as the target contaminants in the current study. Bioavailability of the sediment-associated contaminants was assessed using the bioaccumulation testing using the blackworm, *Lumbriculus variegatus*. Meanwhile, the freely dissolved concentrations of the contaminants in sediment porewater were also measured by the two passive sampling techniques including matrix-solid phase microextraction (matrix-SPME) and polyethylene membrane (PE). In addition, uptake kinetics of the contaminants to the SPME fibers and PE membranes were also estimated in water-only testing. Results showed pyrethroids were less bioavailable compared to their PCB counterparts which had similar Kow values, indicating molecular size played an important role on bioavailability. Similarly, sediment-water partition coefficients (Koc) of pyrethroids were higher than those of PCBs while lower elimination rates were noted for pyrethroids compared to the PCBs. When molecular size was taken into consideration, a better relationship between Kow and Koc was achieved across chemical classes.

**MP100 Biological Effects of Carbon Amendments in Sediment-dwelling *Lumbriculus variegatus*: Implications for Bioaccumulation Testing and Remediation** J. Akkanen, Univ of Eastern Finland, Dept of Biology; I. Nybom, Univ of Eastern Finland; M.T. Leppanen, Univ of Eastern Finland, Dept of Biology; J.V. Kukkonen, Univ of Eastern Finland, Joensuu Campus, Dept of Biology. Different types of amendments have recently been introduced as a potential remediation method for contaminated soils and sediments. In the case of hydrophobic organic contaminants (HOCs), activated carbon (AC) appears to be the most pre-eminent material studied for this purpose. Past studies have heavily focused on the potential of added AC to reduce availability of HOCs. This is first and an important step for testing if the remediation by AC amendment has any chances to meet its goals i.e., to reduce availability of HOCs for transport and bioaccumulation effectively. Less effort has been invested to study direct biological effects of



AC on organisms, which has importance for both laboratory-scale bioaccumulation testing and field-scale ecological impact assessment. In this study, the goal was to assess possible biological responses in *Lumbriculus variegatus*, a widely used test organism in sediment ecotoxicology, to AC amendment in sediments. We tested the effects of AC particle size and manipulation (boiling, solvent extraction) on feeding, growth and reproduction. The results showed that already low doses of AC decreased feeding and retarded growth of the test organisms. Smaller particle sizes caused the effects at lower doses than the bigger sizes. Reproduction proved to be less clear end point, because the test organisms appeared to reproduce also as a response to stress. Boiling and solvent extraction of the AC had little or no effect on the measured responses. Sediment quality (clean natural sediment, clean artificial sediment, PCB-contaminated sediment) had some effect on the responses caused by the added AC. Changed behavior (avoidance) and reduced growth are important factors when assessing bioaccumulation and ecological quality of the sediment. Therefore, we may need to consider also the possible negative effects of carbon amendments when selecting and evaluating site-specific remediation actions.

**MP101 Measuring Remedial Effectiveness of Activated Carbon Amendment in Grasse River, NY** B. Beckingham, Univ of Maryland Baltimore County, Civil & Environmental Engineering, Univ of Maryland Baltimore County, Dept of Civil & Environmental Engineering; U. Ghosh, Univ of Maryland Baltimore County, Civil & Environmental Engineering. Legacy toxic pollutants such as polychlorinated biphenyls (PCBs) persist in sediments and bioaccumulate in the aquatic food web. Recent work has shown that uptake in the food web is strongly influenced by the nature of contaminant binding, especially to black carbon surfaces in sediments. We demonstrate for the first time using multiple lines of evidence that activated carbon amendment to sediments in a contaminated river reduces bioavailability of PCBs. After treatment with activated carbon applied at a dose similar to the native organic carbon of sediment, bioaccumulation in freshwater oligochaete worms measured in both laboratory and field exposures was reduced by 62-99% and concentrations of PCBs in water at equilibrium with the sediment by >95% compared to measurements with unamended background sediments in each year. In this presentation, however, we will focus on the information added by surrogate bioavailability measurements, namely, field and laboratory applications of polyoxymethylene (POM) passive samplers, and sediment desorption kinetics. Passive samplers showed that the chemical activities in pore waters of treated sediments were reduced in comparison to untreated sediments, indicating a lower potential for contaminant flux to the overlying water column. Also, the extent of the reduction in passive sampler uptake at the sediment-water interface in the river was similar to the change in bioaccumulation in field exposures of organisms; therefore, passive samplers may be used as a surrogate to monitor change in chemical availability following remediation. Passive sampling tools can potentially provide more spatial coverage than is practical when employing exposures of live organisms. Desorption kinetics tests showed the rapidly desorbing fraction of PCBs from sediments was reduced following AC amendment, which provides evidence of the mechanism for reduced bio-uptake. We can additionally use these measures of bioavailability to attempt to predict bio-uptake in model organisms. Although aggressive remedies may be appropriate for some highly contaminated sites, we show through this pilot study that PCB exposure from moderately contaminated river sediments may be managed effectively through activated carbon amendment in sediments.

**MP102 Synthesis of Nano-Zero Valent Iron in Sand Matrix and the Impact on Microbial Community** V. Roland, Tennessee State Univ, Dept of Civil and Environmental Engineering; T. Byl, US Geological Survey, Dept of Interior, Tennessee State Univ., College of Engineering. Little is known about the synthesis of nano-sized particles in sand matrices, and the effects on groundwater bacteria. After conducting literature research on the synthesis of nano-zero valent iron, this study was designed to synthesize the nano-particles in a saturated soil matrix. Nano-iron instantly precipitated in the groundwater solution containing 5 ml of 0.5 M NaBH<sub>4</sub> and 1 ml of 0.018 M FeSO<sub>4</sub>. Additional batch reactions were conducted using 100 mL of saturated aquifer soil collected from a field site contaminated with carbon tetrachloride. Initial CCl<sub>4</sub> concentrations in the porewater ranged from 3.8 – 3.9 ppm, which was consistent with the concentrations observed in water samples collected at a nearby spring. Samples were collected from the batch reactors before the addition of iron and NaBH<sub>4</sub>, then 30 minutes, 60

minutes, and 20 hours after the addition of NaBH<sub>4</sub>. Calculations showed that the reaction produced 0.24 L of H<sub>2</sub> gas as seen through production of tiny bubbles. Gas chromatography was used to measure the carbon tetrachloride, chloroform and methylene chloride. Water samples with no NaBH<sub>4</sub> retained 80% of their initial CCl<sub>4</sub> concentration over a 20 hour incubation, 65% of the initial CCl<sub>4</sub> concentration was retained in soil-water reactors. Preliminary results indicated that dechlorination of CCl<sub>4</sub> was occurring in soil and water batch reactors treated with NaBH<sub>4</sub>. Calculations show samples treated with NaBH<sub>4</sub> had 1<sup>st</sup>-order reductive dechlorination reaction rates from 0.04 – 0.1. Additional testing found that the indigenous microbial communities were still healthy despite boron and hydrogen release. The surviving bacteria were fermenting and sulfur-reducing bacteria, which are both important to biological reductive dechlorination. This study showed that nano-zero valent iron particles can be synthesized in saturated soils and reduce halogenated solvents with minimal harm to the geo-bacteria.

**MP103 Antimicrobial Resistance to Veterinary Drugs in the Korean Animal Farm Environment** J. Kwon, S. Lim, National Veterinary Research & Quarantine Service; H. Yun, College of Veterinary Medicine, Chungnam National Univ.; K. Lee, Dept of Applied Biology & Chemistry, College of Agriculture & Life Science, Chungnam Nat'l Univ. Due to the biological activity of veterinary drug residue in the environment, antimicrobials are potential micropollutants. Furthermore, antimicrobial resistance to antibiotics has increased every year in Korea. This study describes antimicrobial resistance in the Korean animal farming environment. Soil, manure compost, liquid fertilizer and water were collected from 4 swine and 12 chicken farms, located in southern province in Korea, from September to October in 2009. *E. coli*, *Enterococcus faecalis*, and *E. faecium* were isolated animal farm environment. Tetracycline resistances were mainly found in pig farms and amoxicillin resistances were mainly found in chicken farms. In case of *E. coli* from pig farm soil showed relatively highly occurrence of various antimicrobial resistances. *E. faecium* from compost of two pig farms presented diverse occurrence of antimicrobial resistance against penicillin, erythromycin, tetracycline, ciprofloxacin, rifampin etc. Furthermore, relationship between the occurrence of residue and the occurrence of antimicrobial resistance was also identified, for example, when tetracycline residues were determined, tetracycline resistant bacteria were always emerged.

**MP104 Dissolved PCB Concentrations in Lotic and Lentic Environments Measured by Passive Samplers** S. Reller, Clemson Univ, Environmental Engineering and Earth Sciences, 342 Computer Court; V. Dang, Clemson Univ, Environmental Engineering and Earth Sciences; D. Delach, Clemson Univ, Environmental Toxicology; C.M. Lee, Clemson Univ, Environmental Engineering and Earth Sciences Dept. In recent years, passive samplers have been employed to monitor and increase our understanding of concentrations of dissolved hydrophobic contaminants in aquatic systems. Results from both dynamic systems (e.g., streams and rivers) as well as more quiescent water bodies (e.g., lakes and reservoirs) have been reported widely. But no comparisons have been made from watersheds with both lotic and lentic components. Passive samplers made of polyethylene (PE) were deployed in a stream in the headwaters of a watershed contaminated with polychlorinated biphenyls (PCBs) and also in a large reservoir in the watershed. More than 200,000 kg of PCBs were released to the watershed from about 1955 to 1975 during operation of a capacitor manufacturing plant. The sediments in tributaries and the reservoir (Lake Hartwell, SC, USA) are part of a Superfund site that has been well studied. The PE samplers (2.5 × 5 cm) were attached to stationary posts in the stream for 60 days. Currently research is underway to deploy PE samplers (2.5 × 12 cm) in the reservoir for 90 days. The samplers were extracted by dialysis in methylene chloride, solvent exchanged, and analyzed by gas chromatography with electron capture detector (GC-ECD) to determine concentrations of up to 140 PCB congeners. Total PCB concentrations ranged from 748.86 ± 191.51 (±SD) ng/g PE to 3547.89 ± 475.96 ng/g PE in the stream. Comparisons between the two aquatic environments will be discussed to understand differences in up-take.

**MP105 Measuring Soil Oxidation-Reduction Potential in a Patchwork World** S.C. Pierce, R. Kroger, Mississippi State Univ, Wildlife, Fisheries, and Aquaculture; R. Reese, C. Griffin, A. Turner, Mississippi State Univ, Electrical and Computer Engineering. In soils and sediments subjected to regular inundation, oxidation-reduction potential (Eh) ranks equal in

importance with more familiar physicochemical soil parameters such as pH, organic carbon, and particle size. Eh, however, experiences temporal fluctuations due to hydrologic variability, temperature, and biotic interactions. Spatial heterogeneity occurs at multiple scales in both in the horizontal and vertical plain. Conventional methods developed for measuring Eh are unable to adequately quantify this inherent variability. Herein, recent advances toward capturing this variability are reviewed, highlighting newly developed field-deployable instrumentation for sampling and recording long-term Eh at short time-intervals and multiple spatial scales. The system, collaboratively developed as a senior engineering project, uses multiple probes and data loggers that have the capability to communicate wirelessly with a central recording device, vastly decreasing sampling effort. Data from tests in the laboratory and field are presented in the context of regulating chemical exchanges of soil and water via hydrologic management.

**MP106 Residence Time Distribution Model For Non-ideal Flow Derived From Independent Gamma Distributions of Tracer Travel Distance and Linear Velocity** L. Embry, Tennessee State Univ; T. Byl, US Geological Survey, Dept of Interior, Tennessee State Univ., College of Engineering; R. Painter, Tennessee State Univ, College of Engineering, Technology and Computer Science. The Residence Time Distribution (RTD) is the time that a particle will take to complete its path from the injection to the exit point in a closed system. Most RTD models have used the advection dispersion equation (ADE) for tracer breakthrough curves for karst systems. ADE solutions for tracer breakthrough curves exhibiting near plug flow behavior are usually Gaussian (normally distributed) in nature. This symmetric solution often predicts finite tracer concentrations at zero time and this is not often shown in tracer breakthrough curves, which frequently are characterized by relatively long upper tails. There are few quantitative tracer studies modeling karst systems that have tracer concentrations normally distributed about the mean residence time and few of these models graphically display this distribution. The objective of this project was to create an M-file language computer algorithm in MATLAB® Version 6.5.1 and GNU Octave Version 3.2.4 that combined both the numerical and visual aspects of karst tracer studies. This project used the transformed residence time distribution function using the independent gamma distributions of tracer travel distance and linear velocity. This algorithm computes the transformed RTD function from the time inputs and then displays the RTD function versus Time graph, the Tracer Concentration versus Time graph, and the graph of the tracer concentration normally distributed about the mean residence time.

**MP107 Using Disposable Polydimethylsiloxane Fibers to Determine the Freely Dissolved Concentration of Polybrominated Diphenyl Ethers (PBDEs) in Sediment** E. Jia, Univ of California, Riverside, Dept of Environmental Sciences, UC Riverside, Dept of Environmental Sciences; J. Gan, Univ of California Riverside, Environmental Sciences. Polybrominated diphenyl ethers (PBDEs) have found extensive use as flame retardant additives. Their long persistence and endocrine disrupting potency have raised concerns about their environmental fate. Sediment is the primary sink for PBDEs, where bioavailability likely regulates the exposure of PBDEs to aquatic organisms. The freely dissolved concentration ( $C_{free}$ ) of HOCs is considered to be a good indicator of bioavailability. However, strong sorption of PBDEs to DOM in sediment porewater presents a great challenge to measuring  $C_{free}$ . In this study, a solid-phase microextraction (SPME) method was developed for determining  $C_{free}$  of PBDEs in sediments using disposable fibers coated with 35  $\mu\text{m}$  of polydimethylsiloxane (PDMS). We carried out concurrent SPME and traditional solvent extraction to evaluate phase partitioning of PBDEs in several sediments. Substantial association of PBDEs with DOM was observed in the sediment porewater. The  $C_{free}$  values in sediment porewater were further used to improve the measurement of sorption coefficient KOC and KDOC of PBDEs.

**MP108 A Method Based on Florisil Column Cleanup for Analysis of Non-ortho PCB Congeners in Rat Adipose with Exposure to Aroclor 1254** N. Li, N. Cekic, W.J. Bowers, I. Chu, Health Canada, Hazard Identification Division, Environmental Health Science and Research Bureau, HECSB. Non-ortho substituted coplanar polychlorinated biphenyls (PCBs) are highly toxic and have extremely low concentrations in commercial PCB formulations such as Aroclor 1254. Quantitative analysis of the coplanar PCBs with GC need separation of the ortho substituted PCBs from the sample prior to injection because the latter may be thousands times higher in concentration and may interfere with their detection. Florisil was studied

as adsorbent to isolate coplanar PCBs from extracts of adipose of laboratory rats with exposure to Aroclor 1254. The retention of 35 representative coplanar and non-planar PCBs and similar persistent chlorinated compounds such as organochlorine pesticides was studied. Florisil (60-100 meshes) was activated at 200°C for 18 hours. The coplanar PCBs have good retention on the adsorbent and at least five of them (PCB77, 81, 126, 127, 169) can be separated from the ortho substituted PCBs with a proper cut point. Moreover, oxychlordane,  $\gamma$ -BHC and cis-Nonachlor have similar retention on the Florisil column with above five coplanar PCBs and were used as internal and surrogate standards for analysis calibration and quality control respectively, and were added to the sample before extraction. In comparison with porous graphite carbon which is widely studied for separation of the coplanar PCBs from the non-planar PCBs, one of the advantages of the Florisil column is that for biological samples the lipid can be simultaneously separated. With a column of 8.0 g of Florisil, up to 0.5 g of lipid could be retained and separated from the sample, and the ortho-substituted PCBs were eluted out from the column with 220 mL hexane, and the five coplanar PCBs were then eluted from the column with 50 mL of 30% DCM in hexane. The problem of a method with Florisil column may be found for the minor carryover of the non-planar PCBs when a stronger solvent is used to elute the coplanar PCBs. By using the internal standard and surrogate standards, this can be solved with a second or third separation with a small size of 1g Florisil column so that PCB153 which is major PCB component in commercial formulations and environmental samples is not detected. The method was validated with reagent blank, method detection limit, recovery and relative standard deviation of replicate analysis, and applied for analysis of the rat adipose samples from toxicological study in our laboratory.

**MP109 A Non-lethal Approach to Assess and Monitor Mercury Concentrations in Black Basses from a Mercury Impacted Stream in the Shenandoah Valley, Virginia** J. Collins, G. Murphy, J. Flanders, URS Corporation. A non-lethal sampling approach utilizing dermal biopsy punches has been developed to assess and monitor total mercury (THg) concentrations in black basses along 26 miles of the South River, Virginia. Fish tissue biopsy samples provide data on spatial, temporal and ontogenetic variations of THg in the muscle tissue of smallmouth and largemouth bass, without causing mortality. Fish were collected through the use of boat and tote-barge mounted electrofishers and held in flow-through live pens prior tissue sampling to minimize handling stress. Tissue samples from up to 30 bass from each biological monitoring location were collected with a mid-dorsal biopsy plug obtained using a 3.5 millimeter (mm) sterile biopsy punch. After sampling a small passive integrated transponder (PIT) tag was inserted beneath the posterior dorsal fin terminus in order to track THg levels in unique individuals over time. Antibacterial salve was applied to the wounds to prevent infection and field observations from the 2009-2011 sampling events indicated that recaptured smallmouth and largemouth bass had complete regrowth of skin and scales between sampling events. Sample results were found to be comparable to traditional lethal sampling techniques and present a sustainable alternative for assessing metals concentrations in fish.

**MP110 An Integrated Screening Methodology for Concise Site Decision Making and Chemical Characterization** J. Guerrero, Space and Naval Warfare Systems Center; R. Johnston, US Navy, Marine Environmental Support Office-NW; J. Leather, Space and Naval Warfare Systems Center; J. Brandenberger, Pacific Northwest National Laboratory; B. Beckwith, Puget Sound Naval Shipyard & IMF; D. Leisle, Naval Facilities Engineering Command – NW. Risk assessments for sediment sites require decisions based upon thoroughly defensible, high quality, analytical data. An integrated screening methodology in combination with high resolution laboratory analytical support is a practical approach to provide for concise site decision-making while balancing data quality needs and cost effectiveness. The Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS&IMF) in Bremerton, WA consists of industrial, commercial, and ship-support facilities that are regulated under the NPDES program and offshore sediments that are being remediated as part of an active CERCLA clean up. The presence of several divergent classes of chemical contaminants, including metals, PAHs and PCBs, presented particular challenges for chemical characterization. During 2010, screening tools were used as part of a tiered management strategy involving an initial screening assessment followed by traditional laboratory analyses. Within ten working days, over 100 sediment samples were rapidly screened for metals (Cu, Zn, Pb, Fe, etc.) by Field Portable X-ray Fluorescence (FPXRF) and organics (PAHs and PCBs)

using Enzyme Linked Immuno-Sorbent Assay (ELISA). A subset (20%) of the total samples were analyzed by more expensive analytical techniques including ICMP-MS for metals and GC/MS for organics. The high density screening data was able to quickly identify pockets of elevated contamination for follow on investigations and confirmation using more expensive methods. The screening tools provided rapid results that supported near real-time decision-making and resulted in assessment objectives that were more focused and tailored to conditions at the site. The screening results coupled with quantitative laboratory analysis also supported water quality assessments under the Clean Water Act 303(d) listing procedures and compliance with Washington State Sediment Quality Standards.

**MP111 Application of Continuous Low-level Aquatic Monitoring Devices to Analysis of Organic Contaminants in Stormwater Retention Ponds on Kiawah Island, SC** L.J. Macaulay, G.J. Getzinger, Duke Univ, Nicholas School of the Environment; B. Hepner, Aqualytical Services Incorporated; L. Ferguson, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Nicholas School of the Environment, Pratt School of Engineering, Dept of Civil & Environmental Engineering. Assessment of trace environmental organic contaminant levels in surface waters has typically been accomplished by grab sampling, composite sampling, or passive sampler deployment. Grab samples offer only a snapshot of concentrations at the time of sampling, while composite samples allow integrated sample collection over a defined time interval, however sample degradation during storage is a concern. Passive samplers (POCIS and SPMD) offer continuous sampling of organic contaminants, but relating the contaminant levels in the passive samplers to the ambient water concentrations can be challenging. In the current work, we have applied an integrative, real time solid-phase extraction technology, termed Continuous Low-level Aquatic Monitoring (CLAM, Aqualytical Services Incorporated, Maple Valley, WA) to assess the levels of thirty polar organic microcontaminants in golf-course retention ponds on Kiawah Island, South Carolina. CLAM devices are novel, in situ extraction samplers consisting of high-flow filtration media and Waters OASIS HLB discs packaged together in a 47 mm syringe filter format. Sample water is drawn continuously through the media using a peristaltic pump, allowing low flow (3-4 mL/minute), integrated sampling. Sources of microcontaminants to the ponds on Kiawah Island included land application of treated municipal wastewater as an irrigant for turfgrass as well as routine application of turf-management chemicals. Analytes thus included wastewater-derived xenoestrogens (e.g., ethynylestradiol, nonylphenol, octylphenol, and bisphenol A) as well as fungicides, pesticides, & herbicides. CLAM devices were deployed at five sampling locations on Kiawah Island for 24 hour intervals every 2<sup>nd</sup> day for two weeks, and extracted water volumes were recorded. The OASIS CLAM extracts were analyzed for microcontaminants by ultra performance liquid chromatography (UPLC) with electrospray high-resolution mass spectrometry detection (HRMS, LTQ-Orbitrap Velos). Comparisons were made between concentrations of analytes in CLAM extracts and those measured in discrete grab water samples via conventional, cartridge-based SPE methods in order to assess the reliability of CLAM samplers as field-deployable, integrated contaminant sampling devices. Results to date indicate that CLAM devices hold great promise for assessment of ambient micropollutant concentrations in contaminated surface waters.

**MP112 Characterization of Tracer Chemicals to Describe Marine Debris Ingested by Hawaiian Seabirds** E. Nilsen; B. Jensen. Seabirds and various other marine organisms ingest plastic fragments on a regular basis as they mistake it for natural food. Previously, the ingested plastics have been characterized based on their physical properties (foam, line etc). There are also abundant amounts of small plastic fragments that are too small to be described by physical properties and typically generically described as "fragments". We developed a method to characterize the ingested marine debris according to the Society of Plastics Industry (SPI) Types (#1-6). Known plastics were analyzed by GCMS for "tracer chemicals" characteristic to each type of plastic (1-6). The tracer chemicals were used as identifiers in the analysis of ingested plastic debris. Retention time and mass spectra were used as the basis for identification. Bolus contents from Laysan albatross at Kure Atoll during the breeding season of 2005 were collected and analyzed. 200 fragments were taken from the total lot to be analyzed by this approach: 129 Fragments, 44 pieces of Foam, 10 pieces of Line, 6 pieces of Sheet and 11 Nurdles. Of the ingested plastic debris analyzed, 1% was comprised of High Density Polyethylene (Type 2), 4% was comprised of Polystyrene (Type 6), 11% was comprised of Polyethylene Terephthalate (Type 1), 20%

was comprised of PolyVinyl Chloride (Type 3) and 64% was comprised of Polypropylene (Type 5). The most abundant type of plastic found is a type that cannot currently be recycled. Using this method to further identify the ingested plastic debris can be useful in determining the type of plastic that is most frequently ingested by various species of marine organisms, as well as the plastic that is the most common contributor to marine debris. Identification of these plastics, in this case Polypropylene (Type 5) and PolyVinyl Chloride (Type 3) can be the basis for redirected Point Source Reduction efforts to limit the amount of plastic debris that enters the ocean environment.

**MP113 Determination of Thyroid Hormones in the Brain and Thyroid Gland Using Isotope Dilution LC-MS/MS** T. Kunisue, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany, Research Scientist, New York State Dept of Health, Research Scientist; K. Kannan, Wadsworth Center, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany; J.W. Fisher, Dept of Environmental Health Science, College of Public Health, Univ of Georgia. Thyroid hormones (THs) play critical roles in the regulation of growth and development, including brain development. Until recently, TH levels were assayed with measurements in serum, using immunoassay (IA)-based methods. IA methods are sensitive but are compromised by the lack of adequate specificity. Further, measurements of TH levels in blood do not necessarily reflect the levels and profiles found in critical organs such as the brain. In this study, we developed a selective and sensitive method for the analysis of six THs: L-thyroxine (T<sub>4</sub>), 3,3',5-triiodo-L-thyronine (T<sub>3</sub>), 3,3',5'-triiodo-L-thyronine (rT<sub>3</sub>), 3,5-diiodo-L-thyronine (3,5-T<sub>2</sub>), 3,3'-diiodo-L-thyronine (3,3'-T<sub>2</sub>), and 3-iodo-L-thyronine (3-T<sub>1</sub>) in the brain and thyroid gland (TG), using isotope (<sup>13</sup>C-T<sub>4</sub>)-dilution liquid chromatography (LC)-tandem mass spectrometry (MS/MS). Proteins in the (rat) brain and TG were digested by pronase, and THs were extracted with a solid-phase extraction (SPE) method and analyzed by LC-MS/MS. The instrumental calibration range for each TH ranged from 0.5 to 200 ng/mL and showed excellent linearity (r>0.9995). The instrumental detection limits for THs were in the range of 7.5-13.5 pg, in positive-ion mode, and 13.5-16.5 pg, in negative-ion mode. The optimized procedural recoveries for THs (except for 3-T<sub>1</sub>), spiked into a brain matrix, were between 97.6 and 109%, with a coefficient of variation (CV) of 1.2 to 8.2%, for the brain, and between 96.4 and 101%, with a CV of 1.8 to 8.6%, for TG. Concentrations of THs in the brain and TG of the five rats were: 2.20-3.65 ng/g T<sub>4</sub>, 1.56-2.20 ng/g T<sub>3</sub>, and below the limit of detection (LOD) for other THs, in the brain; 88.9-274 ng/mg T<sub>4</sub>, 14.1-49.1 ng/mg T<sub>3</sub>, 2.79-12.0 ng/mg rT<sub>3</sub>, 0.176-0.535 ng/mg 3,5-T<sub>2</sub>, 0.340-0.880 ng/mg 3,3'-T<sub>2</sub>, LOD for 3-T<sub>1</sub>, in TG. This method can permit more comprehensive evaluation of TH homeostasis in the brain and other critical organs following exposure to environmental contaminants.

**MP114 Enhanced Pressurized Liquid Extraction Technique for Dioxins, Furans, and Dioxin-like PCBs from Sediments** L. Aguilar-Lazaro, Baylor Univ, Environmental Science; S. Usenko, Baylor Univ, Dept of Environmental Science, Baylor Univ, Assistant Professor. The extraction of organics from environmental matrices typically requires solid-liquid extraction followed by a series of cleanup techniques. USEPA methodologies 1613 and 8290A, describing the analysis of polychlorinated dibenzo-*p*-dioxins and furans from sediment, involve Soxhlet or pressurized liquid extraction (PLE) followed by several packed column and gel permeation chromatography steps. Combining the necessary extraction and cleanup techniques into a single step reduces labor, time, and solvent volume associated with sample preparation and may potentially improve the overall method's recoveries and precision. An enhanced PLE technique was developed for analyzing dioxins, furans, and dioxin-like polychlorinated biphenyls from sediments. The method was developed and validated using standard reference material 1944, as well as sediment samples collected from the Houston shipping channel San Jacinto River waste pits superfund site, TX. Target analytes were quantified using high-resolution gas chromatography/electron impact negative chemical ionization mass spectrometry. Target analyte recoveries and sample preparation costs were compared to EPA method 1613.

**MP115 Environmental Residue of Veterinary Drugs in Korea** J. Kwon, National Veterinary Research & Quarantine Service; H. Yun, College of Veterinary Medicine, Chungnam National Univ.; K. Lee, Dept of Applied Biology & Chemistry, College of Agriculture & Life Science, Chungnam



Nat'l Univ. Environmental contamination through the excretion of animal faeces and urine, and the subsequent dispersion of contaminated manure onto land is an issue of great concern in Korea. Subsequently, the potential exists for quantities of these drugs to be excreted as the parent compound and/or metabolites and enter the environment due to the spreading of manure and slurry on agricultural land, or direct deposition by grazing livestock. This study describes the residue of chlortetracycline (CTC), oxytetracycline (OTC), sulfamethoxazole (SMTZ), sulfamethazine (SMT), and sulfathiazole (STZ), mainly used veterinary drugs in Korea, from animal farming environment. Samples water were collected from 4 swine and 12 chicken farms, located in southern province in Korea, from September to October in 2009. Residue concentration (ng/g) were followings: STZ 0.06-0.23, and CTC 0.17-2.05 in swine farm soil, STZ 0.3-0.5, SMTZ 0.01-0.68, SMT 0.19-0.68, OTC 0.2-0.8, and CTC 0.1 in solid manure compost, STZ 0.010-0.042, SMT 0.017-15.886, and OTC 6.704-6.927 in liquid fertilizer and STZ 0.004, SMT 0.004-0.008, SMTZ PQL-0.006, and CTC 0.008-0.016 in ground water from swine farms. STZ 0.09-0.10 SMT 0.08-0.11, SMTZ PQL-0.07, CTC 0.15-0.16, and OTC 0.09-0.22 in chicken farm soil, STZ 0.3-0.4, SMTZ 0.01-0.16, and CTC 0.18 in chick manure compost, and CTC 0.007 in ground water.

**MP116 Evaluation of Magnetic Hypercrosslinked Resins for Rapid Solid-phase Extraction of Antibiotics** J. Qiu, Shanghai Jiao Tong Univ, School of Life Sciences & Biotechnology; Q. Zhou, Nanjing Univ, School of Environment. A new magnetic hypercrosslinked polymer has been tested as a sorbent for the rapid solid-phase extraction (SPE) of several antibiotics from water samples. The resins had large specific surface area (more than 800 m<sup>2</sup>/g) and hydrophilic character due to the graft of ammonio moieties. This new rapid extraction method was compared to the typical off-line SPE methods using commercially available sorbent Lichrolut® EN column. The results showed that the magnetic resins were consistently better than the commercial sorbent. The recoveries of the antibiotics (tetracycline, chlortetracycline, oxytetracycline, sulfadiazine and sulfamerazine) were all excess 85% by the rapid extraction method. Moreover, the magnetic polymer had a long-term stability when reused for extraction of more than 50 times.

**MP117 Historical Platinum Group Element (PGE) Deposition in Dated Lake Sediment Cores from Lake Ontario and Nearby Small Lakes** F. Yang, Environment Canada; E. Dabek-Zlotorzynska, Environment Canada, Air Quality Research Division; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute; V. Celo, J. Zhao, Environment Canada. The environmental emissions of platinum group element (PGE), particularly platinum (Pt), palladium (Pd), and Rhodium (Rh) have increased enormously in the past 30 years due to their use in automobile catalytic converters and in electronic devices. Landfilling and incineration represent significant pathways in the commercial lifecycle of these elements while open use in automobile catalysts represents a quantifiable pathway to the environment. All these substances are probably entering aquatic systems such as the Great Lakes from waste water treatment plant (WWTP) effluents, road runoff, and atmospheric particle deposition, but the relative extent of contamination from these pathways is unknown. In this study, dated lake sediment cores were used to examine historical PGE deposition in southern Ontario, Canada. Three small lakes located north of the Toronto urban area but undisturbed due their location on private land or in conservation areas, were selected. The input of PGEs to these systems was likely to be primarily from atmospheric deposition. A sediment core from the center of the west basin of Lake Ontario was also examined for comparison with those small lakes. This site is known to be impacted by WWTPs from the surrounding urban area of more than 5 million inhabitants. In this work, PGEs in sediments were measured with a quadrupole inductively coupled plasma mass spectrometer (ICP-MS) after a microwave assisted acid digestion using aqua regia. A cation exchange separation was used to alleviate the matrix-induced spectral and nonspectral interferences prior to ICP-MS analysis. Sediment cores were dated using the 210 Pb dating method. Concentrations and fluxes (ng/m<sup>2</sup>/yr) of Pt and Pd increased in Lake Ontario with flux ratios of 8 and 10 respectively (post-1990 to pre-1900 horizons). Much lower flux ratios for Pt and Pd were found in sediment cores from small lakes (post-1990 to the 1940-50s) and ranged from 0.88 to 3.5. Rhodium showed no anthropogenic enrichment in Lake Ontario sediments, and in small lake sediments it was present at concentrations near its detection limit (~0.4 ug/kg). Compared to small isolated lakes receiving atmospheric deposition,

the higher anthropogenic fluxes of Pt and Pd in Lake Ontario indicate that urban runoff and WWTP effluents are major sources of these PGEs to the Great Lakes.

**MP118 Non-lethal Adipose Sampling of Migratory Songbirds for Persistent Organic Pollutant Analysis** R. Cooper, Arkansas State Univ, Environmental Science; R. Warby, J. Bednarz, Arkansas State Univ. Current techniques for detecting persistent organic pollutants (POPs) in birds are lacking in accuracy and methodology. These limitations prevent us from assessing accurate toxin patterns and trends in birds, ultimately impeding informed conservation. Adipose is the best tissue to sample for accurate determination of pesticide body burdens, often because POPs have high persistence in fat. However, previous fat tissue studies have usually required sacrificing study subjects or salvaging carcasses. This approach results in limited sample size and biased sampling. We have developed a non-lethal method for sampling songbird adipose tissue that allows us to identify and quantify pertinent POPs. To demonstrate the feasibility of this approach we spiked chicken fat with a POP surrogate and separated the fat from the surrogate using a silica gel column. The surrogate concentration was quantitatively determined by gas chromatography/mass spectrometry (GC/MS). This technique was further verified by spiking chicken fat with six polychlorinated biphenyl congeners and an organochlorine mixture. We have applied this analytical method to facilitate monitoring POP concentrations in adipose tissue sampled from migratory songbirds captured at banding stations in northeastern Arkansas and southwestern Ohio. Additionally, potential adverse effects of the sampling method were evaluated in an aviary experiment involving gray catbirds (*Dumetella carolinensis*) and indigo buntings (*Passerina cyanea*). Survivorship, mass change, subcutaneous fat quantity, and fecal glucocorticoid metabolites were evaluated between control and sampled groups.

**MP119 On-line SPE Using Chromolith Guard Columns for Mass Spectrometric Analysis of Urinary Phthalate Metabolites in Exposure Assessment Studies** M. Silva, E. Samada, J. Preau, Centers for Disease Control and Prevention; A. Calafat, Centers for Disease Control and Prevention. Phthalates are a widely used group of chemicals with a spectrum of industrial applications. Because of their wide use, human exposure to phthalates is common. Urinary monoester metabolites of phthalates are generally used for exposure assessment of phthalates in humans. For biomonitoring of phthalates, a number of methods have been reported in the literature. Previously we have developed a method to quantify urinary phthalate metabolites using on-line solid phase extraction (SPE) coupled to high performance liquid chromatography-electrospray ionization tandem mass spectrometry. We modified our method by replacing the SPE column with a second generation chromolith guard column, adjusting the solvent gradient and reducing the SPE wash time. These modifications improved the separation of all analytes, increased the HPLC and SPE column life and resulted in significant cost savings. This method has high selectivity and accuracy with automatic recovery correction, precision and throughput with minimal matrix effects. The limits of detection for all analytes are in the low ng/mL range.

**MP120 One-step SPE Combined with LC-MS/MS for the Simultaneous Determination of 31 Endocrine-disrupting Compounds in Surface Water of Shanghai** H. Zhang, T. Xu, D. Yin, X. Hu, Tongji Univ, College of Environmental Science and Engineering. A novel analytical method employing one-step solid phase extraction (SPE) coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed to detect 31 endocrine-disrupting compounds (EDCs) in surface water samples simultaneously. The target EDCs belong to five classes, including seven estrogens, eight androgens, six progesterones, five adrenocortical hormones and five industrial compounds. In order to simultaneously concentrate the target EDCs and eliminate matrix interferences in the water samples, MCX SPE cartridges were employed for the one-step SPE, and then followed by a simple and highly efficient three-step sequential elution procedure. Two electrospray ionization (ESI) detection modes, positive ion electrospray ionization (ESI+) and negative ion electrospray ionization (ESI-), were optimized for HPLC-MS/MS analysis to obtain the highest sensitivity for all the EDCs. The limit of detections (LODs) were 0.02 to 1.9 ng L<sup>-1</sup> for all the compounds, which are lower than or comparable to these reported in references. Wide linear ranges (LOD-100 ng L<sup>-1</sup> for ESI+ mode, and LOD-200 ng L<sup>-1</sup> for ESI- mode) were obtained with determination coefficients (R<sup>2</sup>) higher than 0.99 for all the compounds. With five internal standards, good

recoveries (84.4% to 103.0%) of all the target compounds were obtained in selected surface water samples. The developed method was successfully applied to investigate the EDCs occurrence in the surface water of Shanghai by analyzing surface water samples from 11 sites. The results showed that nearly all the target compounds (30 in 31) were present in the surface water samples of Shanghai, of which three industrial compounds (4-t-OP, BPA, BPF) showed the highest concentrations (median concentrations were 11.88 ng L<sup>-1</sup>-23.50 ng L<sup>-1</sup>), suggesting that industrial compounds were the dominating EDCs in the surface water of Shanghai, and much more attention should be paid to these compounds. Our present research demonstrated that one-step SPE with MCX cartridges combined with HPLC-MS/MS was convenient, efficient and reliable for multiclass analysis of EDCs in surface water.

**MP121 Passive Dosing as a Speciation Tool** V. Gouliarmou, Aarhus Univ- National Environmental Research Institute, Environmental Chemistry and Microbiology, Aarhus Univ, Environmental Chemistry and Microbiology; K.E. Smith, Aarhus Univ- National Environmental Research Institute; L.W. de Jonge, Aarhus Univ- Faculty of Agricultural Sciences, Agroecology and Environment; P. Mayer, Aarhus Univ- National Environmental Research Institute. The main feature of passive dosing is control of the freely dissolved concentrations by partitioning from a pre-loaded polymer. In the present study we apply this for the development of a new analytical tool to study speciation and binding of hydrophobic organic compounds (HOCs) in aqueous solutions. Further, passive dosing was applied to several medium constituents covering a wide range of interactions with HOCs. The first step of the new method is to control the freely dissolved concentration of HOCs and the second is to measure the total HOCs concentration in the equilibrated aqueous solution (=C<sub>total</sub>). The measured concentration in the solution can then be combined with the corresponding equilibrium partitioning concentration in pure water (=C<sub>water</sub>) to determine: 1) speciation properties of aqueous samples ( $f_{ff} = C_{water}/C_{total}$ ) 2) partitioning of HOCs with dissolved phases e.g., cyclodextrin, humic acids, micelles for determination of K<sub>DOC</sub> and K<sub>D</sub> values 3) the enhanced capacity (E) of solutions for HOCs ( $E = C_{total}/C_{water}$ ) and 4) salting-out effects (Setschenow constants). Application of passive dosing for speciation and binding studies has a number of advantages: 1) it is a precise and simple method, 2) it requires no phase separation step and no mass balance assumption, 3) binding constants can be determined with simple linear regression, 4) it has short equilibration times and reduced fouling problems and 5) it is possible to dose mixtures at defined and environmentally relevant free concentration.

**MP122 Reconstruction of Contaminant Profiles in Marine Ecosystems Using Whale Earwax Plugs Matrices** E. Robinson, Baylor Univ, Environmental Science, Baylor Univ, The Institute of Earth, Ecological, and Environmental Sciences, Baylor Univ, graduate student; S. Trumble, Baylor Univ, Dept of Biology; S. Usenko, Baylor Univ, Dept of Environmental Science, Baylor Univ, Assistant Professor. Using a recently developed analytical method for quantifying semi-volatile organic compounds in whale earwax, pesticides and polychlorinated biphenyls (PCB) contaminant profiles were identified in a Pacific gray whale earwax plug layers. The Pacific gray whale's earwax plug collected in 1969 along the western coast of the US was analyzed for pesticides, PCBs, and polybrominated diphenyl ethers using gas chromatography-mass spectrometry in electron capture negative ionization mode. Results from analysis of earwax layers from the older, outer earwax layers to the younger, inner earwax layers displayed a consistent PCB profile over the whale's lifespan (averaging ~ 0.49 ng/g) and a decreasing pesticide profile towards the end of the whale's lifespan (for example, trans-chlordane decreased from 3.50 ng/g – 1.72 ng/g). Additional earwax plugs from a blue whale harvested off the California coast in 2007 were also analyzed.

**MP123 Using Remote Video Monitoring for Cavity-nesting Waterfowl to Minimize Impact and Maximize Exposure and Productivity Associated Measurement Endpoints** M.W. Nadeau, Michigan State Univ Wildlife Toxicology Lab, Animal Science; W.R. Folland, C.A. Grohman, Michigan State Univ Wildlife Toxicology Laboratory; D.L. Tazelaar, J.M. Matousek, R.R. Holem, J.A. Kubitz, CardnoENTRIX; M.J. Zwiernik, Michigan State Univ Wildlife Toxicology Laboratory. Field studies although difficult to perform, provide real interpretable data pertaining to the exposure and adverse effects of environmental contaminants to individuals and subpopulations of exposed wildlife. Laboratory studies with single compounds require

a considerable investment of resources, especially for constituents that are transferred through the food web and may not be predictive of effects in the field where receptors may be exposed to multiple contaminants from a variety of sources and exposure pathways. The life history characteristics of wood ducks (*Aix sponsa*) and hooded mergansers (*Lophodytes cucullatus*) make these species of cavity-nesting waterfowl ideal for monitoring exposures and effects of environmental contaminants (and mixtures) in aquatic ecosystems. These characteristics include diversity of nesting habitat, high trophic status, dietary composition, foraging range, and the opportunistic utilization of artificial nest boxes. In this study we installed nest boxes with inexpensive weatherproof infrared video cameras. The cameras permitted nest boxes to be routinely checked for the presence or absence of adults, clutch and incubation initiation, eggs and nestlings in a fraction of the time it would take to physically climb the tree and accomplish the same task. Still and video imaging adds transparency to data collection and provides gains in quality assurance and quality control. Additionally, remote monitoring decreases potential stress on nesting hens by reducing flush occurrences and damage to trees during nest access. Furthermore, video monitoring adds precision in identifying clutch and incubation initiation dates resulting in greater opportunities for embryo development specific sampling events. Climbing to access the nest boxes can be limited to maintenance and collection of tissues, reducing both the potential for long term damage to nest trees, the chance of injury to tree-climbing personnel, and labor costs. Utilizing weatherproof infrared video cameras to monitor artificial nest boxes decreases costs and stress to study animals thereby increasing the effectiveness of monitoring reproductive success and contaminant exposure of cavity-nesting waterfowl.

**MP124 Occurrence and Profiles of Benzotriazole UV Stabilizers in House Dust from the Philippines** J. Kim, T. Isobe, Senior Research Fellow Center, Ehime Univ; G. Malarvannan, Center for Marine Environmental Studies (CMES), Ehime Univ; K. Chang, Dept of Environmental Science and Engineering, Kyung Hee Univ; A. Sudaryanto, Technology Center for Marine Survey; M. Prudente, Lasallian Institute for Development and Educational Research De La Salle Univ; S. Tanabe, Center for Marine Environmental Studies (CMES), Ehime Univ. Benzotriazole ultraviolet stabilizers (BUVSs) are used in a variety of consumer products, such as plastic resins, paints, varnishes, films, sealants, adhesive agents, air fresheners, waxes, coating materials, and some sports equipments. For the general population, dermal contact, inhalation and ingestion of dust can be considered as the most important pathways of BUVSs exposure. However, the primary routes of human exposure to BUVSs are still unclear. In this study, BUVSs were measured in the house dust samples collected from a residential area (Malate: n = 17) and municipal dumping area (Payatas: n = 20) in the Philippines. We also assessed the human exposure to these contaminants via house dust ingestion. Total BUVSs concentrations in the house dust samples ranged from 2.87 to 1020 ng/g in the residential area and from 2.91 to 277 ng/g in the municipal dumping area. The most abundant BUVSs found were UV-234 and UV-326, with a median value of 83.7 ng/g (ND – 813 ng/g) and 40.7 ng/g (ND – 212 ng/g), respectively. Significantly higher concentrations of UV-326 (p < 0.01) and UV-327 (p < 0.05) were found in house dust samples from Malate than Payatas suggesting that the household appliances are the major sources of the contamination by these analytes. The estimated dietary intakes (EDIs) of BUVSs through house dust ingestion in the Philippines were two to four orders of magnitude lower than the guideline values. However, the EDIs of 7 BUVSs for toddlers in this study was 5 times higher than adults, suggesting that toddlers are at higher risk. To our knowledge, this is the first comprehensive study on BUVSs contamination in house dust samples in a developing country and notably first study in the world for the three BUVSs, UV-9, UV-234 and UV-320.

**MP125 Can Hair Be Used as an Effective Biomarker to Link Environmental Exposure to Health Outcome?** K. Aleksa, Hospital for Sick Children, Dept of Clinical Pharmacology and Toxicology, Univ of Waterloo, School of Pharmacy. Over the last 20 years hair has moved from the arena of being a novel and highly questionable biological matrix to a mainstream and acceptable matrix in forensic science where it is primarily used to determine exposure (past and present) to illicit drugs. The use of hair as a conventional matrix to assess exposure to pesticides and persistent environmental pollutants has not yet become common. The applicability of this matrix to assess an individual's body burden to chemicals such as polybrominated diethyl ethers (PBDEs) can provide more insight into current exposure levels but also to past exposure levels, which is not possible



with more traditional matrices such as blood and urine. Further as it has been shown that PBDEs cross the placenta and since a fetus' hair begins to grow during the first trimester, this matrix can be used to assess an infant's in utero exposure. These features of hair can in turn be used to determine if chemicals such as PBDEs may be responsible for any physiological or anatomical abnormalities in infants, children or adults.

**MP126 Investigation of Flame Retardants in Indoor Dust from New Zealand: Implication for Human Exposure** N. Ali, Antwerp Univ, Pharmaceutical sciences, Toxicology centre; N.V. Eede, A. Dirtu, H. Neels, A. Covaci, Univ of Antwerp, Toxicology Centre; S. Harrad, Univ of Birmingham, Division of environmental health and risk management; A. Mannetje, J. Coakley, J. Douwes, Univ of Massey, Centre for Public Health Research. Due to the worldwide regulations on PBDEs, the demand for alternative flame retardants (FRs), such as organophosphate esters (OPEs) and novel brominated FRs (NBFRs), has increased. We investigated the presence of twelve PBDE congeners, five NBFRs and ten OPs in indoor dust from New Zealand homes. Dust samples were taken from living room floors (n=34) and from mattresses (n=16). The following NBFRs were measured: decabromodiphenyl ethane (DBDPE) (< 5-131 ng/g), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) (< 2-22 ng/g), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) (< 2-22 ng/g) and bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (TBPH) (< 2-81 ng/g). Concentrations of PBDEs ranged from 54-7477 ng/g. The PBDE profile was dominated by BDE 209 (< 5-6971 ng/g), while other congeners were present at lower concentrations (BDE 47 (< 2-100 ng/g), BDE 99 (< 2-220 ng/g), BDE 183 (< 2-65 ng/g)). The following OPEs were present in the NZ dust samples: tri-ethyl-phosphate (TEP) (range < 10-72 ng/g), tri-n-butyl-phosphate (TnBP) (< 20-830 ng/g), tris-(2-chloroethyl)-phosphate (TCEP) (< 20-443 ng/g), tris-(2-chloroisopropyl)-phosphate (TCPP) (20-3650 ng/g), tri-(2-butoxyethyl)-phosphate (TBEP) (50-11800 ng/g), tris-(2,3-dichloropropyl)-phosphate (TDCPP) (20-2090 ng/g), tri-phenyl-phosphate (TPHP) (20-3490 ng/g), tri-cresyl-phosphate (TCP) (< 50-1025 ng/g). Levels of BDE 47, 99, 183 and 209, BTBPE, DBDPE, TBPH, TBEP, and TnBP showed significant correlation ( $p < 0.05$ ) between their concentrations in mattresses and the corresponding floor dust (n=16). Different exposure scenarios were calculated using the 5<sup>th</sup>, median and 95<sup>th</sup> percentile concentrations. Exposure scenarios were calculated assuming 100% absorption of intake dust, 70 kg body weight (bw) for adult and 20 kg bw for toddlers, mean dust ingestion (adults=20 mg/day; for toddlers=50 mg/day) and high dust ingestion (adults=50 mg/day; for toddlers=200 mg/day). Typical high end exposure, using median concentrations and high dust ingestion, estimates for adults ranged between < 0.01-0.38 ng/kg bw/d for PBDEs, < 0.01-0.01 ng/kg bw/d for NBFRs and < 0.01-2.20 ng/kg bw/d for OPEs. By comparison, for toddlers, typical high end exposure fell between 0.04-7.05 ng/kg bw/d for PBDEs, 0.01-0.11 ng/kg bw/d for NBFRs and 0.05-30.5 ng/kg bw/d for OPEs. Exposure assessment values were multifold below their RfD values. However, their presence in indoor dust emphasizes the need to evaluate health implications for human exposure.

**MP127 Predictors of HBCDs and TBBPA in Milk from Boston Mothers** C.C. Carignan, Boston Univ School of Public Health, Dept of Environmental Health; N. Wu, California Dept of Public Health; M. Abdallah, Assiut Univ, Dept of Pharmaceutical Analytical Chemistry; W. Heiger-Bernays, M.D. McClean, Boston Univ School of Public Health, Dept of Environmental Health; S. Harrad, Univ of Birmingham, Division of Environmental Health and Risk Management; T.F. Webster, Boston Univ School of Public Health, Dept of Environmental Health, Boston Univ School of Public, Associate Professor. Hexabromocyclododecanes (HBCDs) and tetrabromobisphenol-A (TBBPA) are flame retardants widely used in consumer products such as building insulation, electronic housing, and circuit boards. Biological measures of HBCD and TBBPA in European populations have been associated with levels in house dust as well as diet, however scant measures exist for North American populations. Our objectives were to characterize levels of these flame retardants in breast milk from Boston mothers (n=43) and identify significant predictors of exposure using questionnaire data. We analyzed samples for TBBPA,  $\Sigma$ HBCDs,  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD, pentabromocyclododecenes ( $\Sigma$ PBCDs) and tetrabromocyclododecadienes ( $\Sigma$ TBCDs).  $\Sigma$ HBCDs and  $\Sigma$ TBBPA were detected in 100% (359-8,100 pg/g-lw; GM: 1,020 pg/g-lw) and 30% (< 25-549 pg/g-lw; GM: 33 pg/g-lw) of milk samples, respectively. These concentrations are similar to those reported in other countries.  $\Sigma$ PBCDs and  $\Sigma$ TBCDs, two possible

metabolites of HBCD, were detected in 42% (< 30-317 pg/g-lw; GM: 35 pg/g-lw) and 56% (< 26-562 pg/g-lw; GM: 49 pg/g-lw) of milk samples, respectively, and are the first reported measurements of these compounds in human samples from the US. Consistent with observations in other populations,  $\alpha$ -HBCD was the dominant diastereomer accounting for 40-80% of  $\Sigma$ HBCDs with a slight enrichment of the (-)- $\alpha$ -HBCD enantiomer. Levels of  $\Sigma$ HBCDs were significantly correlated with the HBCD diastereomers ( $r > 0.91$ ) and metabolites ( $r > 0.74$ ), as well as TBBPA ( $r = 0.56$ ). Average concentrations of  $\Sigma$ HBCDs,  $\Sigma$ PBCDs,  $\Sigma$ TBCDs,  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD were 47 to 71% lower in the milk of mothers who indicated they regularly chose to eat organic foods than those who did not, whereas these levels were 1.2-1.5 pg/g-lw higher for each additional audio-visual appliance in a mother's home ( $p < 0.05$ ). Our results indicate that both diet and indoor exposures may contribute to levels of these flame retardants in human milk.

**MP128 Baseline Monitoring at Sediment Remediation Sites Under the Great Lakes Legacy Act** B. Jones, S. Ireland, M. Loomis, USEPA, Great Lakes National Program Office. The Great Lakes Legacy Act (GLLA) was signed into law in 2002 and was reauthorized in 2008. The purpose of the Act is to provide funding to take the necessary steps to clean up contaminated sediment in "Areas of Concern located wholly or partially in the United States." USEPA's Great Lakes National Program Office (GLNPO) is charged with addressing sediment contamination problems under the GLLA. Since fiscal year 2004, GLNPO and our non-federal sponsors, have spent approximately \$180 million to address sediment contamination on nine separate remediation projects resulting in approximately 1.3 M cubic yards of sediment remediated. GLNPO performed baseline monitoring at eight GLLA projects, seven sites prior to implementation of sediment remediation, one site post-remediation. The sites are located throughout the Great Lakes basin. This presentation will discuss the approach GLNPO is taking to baseline monitoring of the sites. This approach is part of a strategy to develop guidelines for GLLA remedy effectiveness monitoring as a tool to evaluate program effectiveness. This presentation will present the tools and sampling strategies employed and provide a discussion of the proposed next steps in the monitoring program.

**MP129 Hydrologic and Geologic Controls on Carbon Tetrachloride Transport and Biodegradation at the Velsicol Dump, Hardeman County, Tennessee** A.C. West, Tennessee State Univ, Civil & Environmental Engineering. An unlined landfill in Hardeman County, Tennessee was used by the Velsicol Chemical Corporation from 1964 to 1973 to dispose of 100,000 to 300,000 barrels of industrial waste. An investigation of groundwater contamination at the landfill was conducted to identify contaminant loads in the tributaries, contaminant sources, mode of contaminant transport and potential for in situ biodegradation. Seepage was measured and water quality samples collected along the tributaries. The seepage investigation and water-quality results identify a two-phase mode of contaminant transport to the tributaries. A diffuse ground-water plume transports contaminants to the streams resulting in relatively low and consistent concentrations and loads in the tributaries. High permeability zones, probably related to gravel in the terrace deposits, and concentrated flow near sand/clay contacts have produced seeps and springs along the banks of the tributaries and the edge of the adjacent wetland. The seeps and springs show concentrations of carbon tetrachloride ranging from 2 to 28 mg/L; notably higher than contaminant concentrations in the tributaries. Geochemical and microbial evaluations from multiple groundwater samples indicate that conditions are favorable for natural or enhanced attenuation of the contaminants. Groundwater along the tributaries and discharge into the wetlands was iron or sulfur reducing. Lab microcosms set up with aquifer materials had carbon tetrachloride half-lives of 90 days and when supplemented with lactate and molasses  $T_{1/2} = 2$  days. In general, contamination from the landfill continues to migrate from the landfill to a creek to the east, to the Clover Creek wetlands to the north, and to tributaries west of the site. A large contaminant mass remains in the subsurface, and dissolved phase VOCs are being released along the tributaries to Clover Creek and at seeps along the southern edge of the Clover Creek wetland.

**MP130 Monitored Recovery from Historical PCB Releases in Stony Creek, Indiana** D. Pelletier, M.H. Henning, A.L. Fogg, ENVIRON International Corporation; J. Johnson, Bridgestone Americas, Inc. Clearly defined decision rules and quantifiable success metrics are critical to the success of Monitored Natural Recovery (MNR) as an acceptable remedial



alternative at contaminated sites. Based on data from Stony Creek, a polychlorinated biphenyl (PCB)-contaminated stream in central Indiana (USA), this presentation illustrates the importance of designing a monitoring plan with sufficient statistical rigor to determine when recovery goals have been met. MNR was selected as the preferred remedial alternative for Stony Creek in a 2001 Administrative Order on Consent (AOC) between the responsible party and US Environmental Protection Agency (USEPA), Region 5. The original AOC established threshold PCB concentrations in sediment and fish of 1 milligram per kilogram (mg/kg) and 2 mg/kg, respectively, and monitoring occurred on schedule from 2003 through 2007 under an approved monitoring plan. However, small sample sizes rendered it difficult to differentiate results that were slightly less than the threshold concentrations from those that were slightly greater than the thresholds. Similarly, because the species and size classes of fish collected changed over time, the fish tissue data did not allow for any definitive conclusions regarding trends in tissue PCB concentrations over time. Given the limitations of the old monitoring program, a revised plan was submitted to USEPA in August 2009. The new plan, which USEPA approved, expanded the spatial scope and sample sizes for sediment and fish tissue sampling, while focusing on three fish species representing different feeding guilds. Concurrently, decision rules for determining the need for continued monitoring were reworded for clarity. The primary goal of the updated monitoring plan was to maximize statistical rigor and, consequently, improve clarity and certainty in decision making. Following implementation of the revised monitoring plan, it was possible to demonstrate that continued monitoring of sediment and fish filets was not necessary, and that monitoring should focus solely on whole body fish tissue concentrations going forward.

**MP131 Tenax Absorbent as a Measurement of PCB Bioavailability in Sediment: A Good Surrogate for Biota?** E.M. Tripp, Southern Illinois Univ; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology; P.M. Landrum, Southern Illinois Univ; J. You, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy Sciences, State Key Laboratory of Organic Geochemistry. In the past, bioavailability has been estimated using biota-sediment accumulation factors (BSAF) based on equilibrium partitioning theory. The BSAF is the relationship between the concentration in the organism (lipid normalized) and the concentration in the sediment (organic carbon normalized). This relationship has been shown to be unreliable when using field-collected sediments driven mostly by the character of the organic carbon, particularly the amount of "black carbon". Therefore, developing a more accurate method to determine the bioavailable fraction of contaminants in sediment should improve environmental assessments. One possible method to accomplish this is a single 24 h point extraction using Tenax TA resin. The goal of this technique is to use Tenax to estimate the bioaccessible portion of the sediment-associated contaminant to compare to bioaccumulation in organisms. This study examined the relationship between the bioaccessible fraction of PCBs measured by Tenax and the body residues in oligochaetes. Eighteen field-collected sediments from the Ottawa River (OR) were subjected to 14 d bioaccumulation test with *Lumbriculus variegatus* and a 24 h Tenax extraction. This technique provided a rapid processing time with relatively clean samples for analysis. To validate this technique, data from the OR was combined with data from two other studies using similar methods and one utilizing field-collected oligochaetes. When combined, all of the data follow a single log-log regression when using all congeners ( $r^2 = 0.91$ ). Further separation of data into homologue groups produced variable relationships with correlation coefficients ranging from  $r^2 = 0.76$ - $0.95$ . Due to the effectiveness of predicting PCB body residues in organisms Tenax extraction can be used in site evaluations as well as assessing the potential success of remediation efforts.

**MP132 Advances in Phytoextraction of PCBs from Soils in Southern Ontario Using Weed Species** B. Zeeb, Royal Military College, Chemistry & Chemical Engineering Dept; S. Ficko, Royal Military College; A. Rutter, Queens Univ, School of Environmental Studies. Remediation of soil contaminated with low levels of polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs) continues to be a significant issue in Canada and around the world. While traditional remediation methods such as excavation and incineration are effective, they are expensive not always sustainable. Research investigating phytoextraction as an alternative strategy has mainly focused on crop species such as *Cucurbita pepo* ssp *pepo* (e.g., pumpkins), which have shown a tendency to accumulate POPs in shoot

tissues. In 2010, Ficko et al. documented 27 species of weeds that were capable of accumulating PCBs in plant tissues. Of these, 18 species were theoretically able to extract a similar or greater quantity of PCBs than *C. pepo* plants (when planted at an optimal density). Thus this study demonstrated for the first time that weeds may play a useful role in remediation activities. Advantages of using weeds for phytoextraction include that they are easy to cultivate and propagate, generally self-sustaining, relatively inexpensive, and are often hardier than many cultivated species. In a follow-up experiment, three of the most promising perennial species (*Chrysanthemum leucanthemum*, *Rumex crispus*, and *Solidago canadensis*) were planted in monoculture plots at two historically-contaminated and naturally-weathered PCB field sites, and harvested on a monthly basis over two years. Plant age and life cycle affected total phytoextraction on a per-species-basis and trends were similar between sites. Despite the suboptimal planting densities used in this experiment, all three species were able to extract a greater quantity of PCBs per unit area ( $4800$ - $10,000 \mu\text{g}/\text{m}^2$ ) than *C. pepo* plants ( $1500$ - $2200 \mu\text{g}/\text{m}^2$ ). Thus at optimal densities, these species show significant potential to remediate contaminated soil. In addition, analysis along the stem length of *S. canadensis* plants, demonstrated that PCB movement within plant tissues of other species follows a similar pattern as in *C. pepo* plants. Understanding the differences in uptake between species may help elucidate the mechanisms of phytoextraction, ultimately leading to novel remediation strategies that accelerate the attainment of remediation targets.

**MP133 Four Decades of Discharge: The Toxic Legacy of the New Idria Mercury Mine** K. Manheimer, USEPA, Region 9, Superfund Division; K. Abusaba, Brown and Caldwell, Water Resources. The New Idria Mercury Mine is the second largest historic producer of mercury in North America. Located in the Coast Ranges of California in San Benito County, the mine ceased operations in 1972. Since that time, inadequate closure and maintenance activities have caused a continuous discharge of  $10 - 30 \text{ gpm}$  acid mine drainage and erosive mobilization of mercury from a massive pile of discarded calclines and waste rock. The mine site has recently been proposed for addition to the EPA's National Priorities List; if approved, one important task will be to assess mercury toxicity and effects on environmental receptors both locally and in the greater Central Valley watershed. This talk will present what is currently known about the environmental effects of mercury released from New Idria, along with uncertainties that need to be addressed. A considerable amount of existing information is available to support the proposed listing. Process studies of mercury in solid waste sources from New Idria indicate that alteration forms of mercury sulfide ore created by the process of roasting and weathering increase the availability of solid mercury for dissolution and biological uptake. Methylmercury measurements in water indicate unfiltered concentrations ranging from  $1 - 47 \text{ ng/L}$ , confirming that mercury released from New Idria is biologically available to methylating bacteria. Biologically available mercury is transported five miles downstream to a low point where the impacted stream percolates underground during most times of the year, creating a wetland in an otherwise arid landscape. Recent information has shown that during episodic storm events, water can escape the receiving wetland and break through to the Central Valley, transporting bioavailable mercury into a much more extensive ecosystem. Key questions to be addressed in future studies include: (1) are elevated methylmercury concentrations observed in prey items, such as benthic invertebrates, of the receiving wetland? (2) What receptors feed on those prey items (e.g., the nearby Pinnacles National Monument is part of the California Condor Recovery program, and is the home of 30 free-flying California Condors). (3) To what extent does mercury discharged from New Idria affect the food web in the San Joaquin River and the Mendota Pool, which are important fisheries resources for people and habitat resources for wildlife, as well as an important source of irrigation water for the surrounding farmland?

**MP134 Using the Trident Probe to Characterize Chloride Contamination at the Groundwater-Surface Water Interface (GSI)** D. Lavoie, F. Dillon, J. Johnson, G. Dyke, CH2M Hill; B. Chadwick, Coastal Monitoring Associates, Marine Environment Branch. Code 2362; C. Smith, Coastal Monitoring Associates. Historical investigations at an industrial facility in the Midwest identified elevated chloride concentrations in groundwater. In 2006, on- and offsite cone penetrometer testing (CPT)/electrical conductivity (EC) logging and groundwater sampling suggested chloride might be venting into an adjacent lake at concentrations exceeding the state ecotoxicological benchmark. Although EC probes showed indirect chloride

concentration measurements in the lake at the groundwater-surface water interface (GSI) were lower than in deep grab samples, no direct chloride measurements in shallow sediment pore water had previously been collected. Therefore, 2 phases of sediment pore water sampling were conducted to survey chloride venting zones using the Trident Probe, a direct-push, integrated conductivity/temperature (CT) sensor and GSI sampler. Phase 1 (April 2008) was conducted to verify the existing hydrogeological CSM, identify specific venting hotspots and gauge concentration magnitude. Phase 2 (May 2010) was conducted to refine the delineation of Phase 1 hotspots, establish a potential relationship between onsite groundwater and offsite sediment pore water, and support potential corrective action measures. For both studies, the Trident Probe was configured with 2 CT sensors and 3 pore water sample probes. Each probe consisted of a 6" sand-pack pre-filter to minimize potential clogging from fine-grained sediment, and attached to peristaltic pumps to facilitate low-flow sampling. The midpoint of the sample probes were set to penetrate to depths of 4", 16" and 29" below the sediment surface, with CT sensors set to collect measurements within the shallowest and deepest depth ranges. In addition to temperature and conductivity, surface water and pore water was further analyzed *ex situ* for conductivity and temperature as well as pH, ORP, TDS. Split samples were analyzed for chloride both in the field, using titrator test strips for real-time screening purposes, and in the lab for definitive results. Both Phase 1 and 2 results were consistent with the existing hydrogeological CSM. Two venting zones, where chloride exceeded the benchmark at the GSI, were identified and delineated in the nearshore of the lake. Data from these studies clearly established the site-specificity of the sediment-associated chloride source and facilitated a focused path forward for onsite corrective measures.

**MP135 Characterizing Volatile Organic Compound Contamination at the Groundwater – Surface Water Interface (GSI) Using the Trident Probe** J. Johnson, F. Dillon, E. Kroger, D. Boehnker, L. Raterink, D. La-voie, CH2M Hill; C. Smith, B. Chadwick, Coastal Monitoring Associates. Historical investigations at an industrial facility in the Eastern United States identified elevated volatile organic compounds (VOCs) in site groundwater (TCE, PCE, DCE, vinyl chloride, chlorobenzene, dichloropropane, dichloroethane and naphthalene). Recent site-related groundwater data were evaluated to identify potential groundwater plume venting areas in the river adjacent to the facility where ecological receptors might be exposed at the groundwater-surface water interface (GSI). Multiple investigation phases of pore water sampling were conducted to identify potential VOC venting zones and determine if VOC concentrations in sediment pore water potentially pose unacceptable ecological risks to aquatic biota. Each phase of the investigation involved collection of pore water for VOC analysis using the Trident Probe, a direct-push, integrated conductivity/temperature (CT) sensor and GSI sampler. The Trident Probe was configured with two CT sensors and three pore water sample probes. The CT sensors were set to collect measurements within the biologically active zone (shallow sediment) as well as from the surface water. The first phase of the investigation included collection of pore water from 15 nearshore locations opposite areas of elevated onshore groundwater VOCs in 7 of the 9 site remediation areas. The initial phase confirmed the presence of VOCs in pore water in exceedance of ecological screening values opposite 6 of the site remediation areas. Subsequent investigations were designed to facilitate additional pore water characterization in identified venting zones and in areas not previously investigated, to establish plume-specific attenuation factors for application towards refinement of groundwater performance criteria at each onsite remediation area. The Trident Probe data was also used to refine the hydrogeological conceptual site model in regards to the relationship of site groundwater with the GSI. This presentation presents the Trident Probe methods employed, summarizes the pore water and groundwater data and conclusions generated from the study.

**MP136 Hydrologic and Geologic Controls on Carbon Tetrachloride Transport and Biodegradation at an Unlined Landfill, Hardeman County, Tennessee** T. Byl, US Geological Survey, Dept of Interior, Tennessee State Univ., College of Engineering; M.J. Bradley, Smithers Viscent, Sediment Ecotoxicology; A.C. West, Tennessee State Univ, Civil & Environmental Engineering. An unlined landfill in Hardeman County, Tennessee was used by the Velsicol Chemical Corporation from 1964 to 1973 to dispose of 100,000 to 300,000 barrels of industrial waste. An investigation of groundwater contamination at the landfill was conducted to identify contaminant loads in the tributaries, contaminant sources, mode

of contaminant transport and potential for in situ biodegradation. Seepage was measured and water quality samples collected along the tributaries. The seepage investigation and water-quality results identify a two-phase mode of contaminant transport to the tributaries. A diffuse ground-water plume transports contaminants to the streams resulting in relatively low and consistent concentrations and loads in the tributaries. High permeability zones, probably related to gravel in the terrace deposits, and concentrated flow near sand/clay contacts have produced seeps and springs along the banks of the tributaries and the edge of the adjacent wetland. The seeps and springs show concentrations of carbon tetrachloride ranging from 2 to 28 mg/L; notably higher than contaminant concentrations in the tributaries. Geochemical and microbial evaluations from multiple groundwater samples indicate that conditions are favorable for natural or enhanced attenuation of the contaminants. Groundwater along the tributaries and discharge into the wetlands was iron or sulfur reducing. Lab microcosms set up with aquifer materials had carbon tetrachloride half-lives of 90 days and when supplemented with lactate and molasses  $T_{1/2} = 2$  days. In general, contamination from the landfill continues to migrate from the landfill to a creek to the east, to the Clover Creek wetlands to the north, and to tributaries west of the site. A large contaminant mass remains in the subsurface, and dissolved phase VOCs are being released along the tributaries to Clover Creek and at seeps along the southern edge of the Clover Creek wetland.

**MP137 An Integrated Approach to Developing a Total Facility Estrogen Budget at a Swine Farrowing CAFO** E. Yost, North Carolina State Univ, Environmental and Molecular Toxicology; M.T. Meyer, United States Geological Survey, Organic Geochemistry Research Laboratory; B. Lee, Duke Univ; S.W. Kullman, North Carolina State Univ, Environmental and Molecular Toxicology. Naturally occurring estrogens from concentrated animal feeding operations (CAFOs) present an emerging risk to terrestrial and aquatic environments through their potential action as endocrine disruptors. Here, we present our findings on the concentrations, fate, and biological availability of estrogens in a commercial swine farrowing CAFO, which houses 5,000 pregnant and lactating female swine. Analysis of estrogens was made in relation to: 1) reproductive status and estrogen excretion by individual animals; 2) the stability of estrogens in open pit holding lagoons; and 3) attenuation of estrogens following spray field application of swine waste as fertilizer. Yeast estrogen screen results indicate  $17\beta$ -estradiol equivalents (EEQ) in sow urine ranging from 1ng/l to 196 $\mu$ g/l, and in sow feces ranging from 16ng/kg to 208 $\mu$ g/kg (dry weight), with a defined gradient of estrogenic activity observable in relation to the reproductive stage of the animal. LC/MS-MS results indicate that the natural estrogens contained in the raw swine excreta (e.g.,  $17\beta$ -estradiol,  $17\alpha$ -estradiol, estrone, estriol) are largely transformed to estrone during their holding period in the waste lagoon, resulting in estrone concentrations averaging 6.2 $\mu$ g/l in lagoon wastewater liquids and 1.7g/kg (dry weight) in lagoon suspended solids. Phytoestrogens, most notably equol, were also found to persist at high concentrations in the lagoon. Analysis of the CAFO spray field indicates that estrogens from the lagoon wastewater are largely retained in the top 6 inches of soil. Estrone was found to persist in this topsoil at parts-per-trillion concentrations for at least 2 months following the land application of lagoon waste. The end goal of our analysis is to develop a hierarchical structure where assessments of estrogen fate can be "parameterized" and used for input into a Bayesian network model, which will be used to explore and predict the behavior of estrogens in the CAFO system. The anticipated result of this project is prioritization of operational practices in regards to waste management strategies and contributions of total estrogens to the environment.

**MP138 Caged Fish Studies to Detect and Monitor Contaminants of Emerging Concern in the Great Lakes** M. Kahl, G. Ankley, USEPA, Mid-Continent Ecology Division; T. Collette, USEPA, National Exposure Research Laboratory; E. Durhan, USEPA, Mid-Continent Ecology Division; D. Ekman, USEPA, National Exposure Research Laboratory; K.M. Jensen, USEPA, Mid-Continent Ecology Division; K. Lee, US Geological Survey; E. Makynen, L. Thomas, USEPA, Mid-Continent Ecology Division; A. Trowbridge, US Fish and Wildlife Service; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division. Effects-based monitoring studies were conducted in the St. Louis Harbor, Lake Superior, in support of the Great Lakes Restoration Initiative (GLRI). The overall goal of the research was to develop and validate methods using caged fish exposures to detect and monitor contaminants of emerging concern (CECs) around the Great Lakes. In 2010, four field sites including the upper St. Louis River (Fond du Lac),

two locations near the Western Lake Superior Sanitary District (WLSSD), and one location near the Superior Municipal Treatment Plant (SMTP) were investigated. Caged fish exposure systems and integrated water sampling devices were developed, and sexually mature fathead minnows (*Pimephales promelas*) were deployed at each location for 2 to 10 days. A number of endpoints were examined in the fish, including plasma vitellogenin and steroid concentrations, expression of different genes that could be impacted by specific CECs, and NMR-based hepatic metabolite profiles. In addition, an extensive suite of CECs were measured in water from the sites where fish had been deployed. Survival and recovery of the fish from the in situ exposure system was excellent, and initial results suggest the presence of estrogenic and/or steroidogenesis-altering chemicals or conditions at sites proximal to waste water treatment plant discharges. Preliminary chemical analyses indicate substantial difference among the sites with regard to CEC profiles. This presentation will provide an overview and synthesis of the biological and analytical work done to date, as well as recommendations for conducting future studies of this type at other Great Lakes sites. The contents of this abstract do not reflect USEPA policy.

**MP139 Predicting Hormone Concentrations in Lakes Due to Inputs From Wildlife and Fish** E. Osborn, ARCADIS; G. Scheef, Intervet Innovation GmbH; P. Anderson, ARCADIS. Endocrine disrupting chemicals in parts per trillion concentrations have been reported in Minnesota lakes by Writer et al. (2010). Anthropogenic sources for these compounds, such as treated wastewater discharge, are generally assumed. However, some lakes with detectable concentrations of hormones have no known anthropogenic inputs. We developed a mass balance model to predict theoretically possible concentrations of estrogens, androgens, and progestagens in pristine northern lakes due to natural inputs from wildlife and fish. Real lakes were modeled, and inputs from mammals, birds, and fish were estimated using excretion rates from the literature and estimated population parameters. Chemical loss was assumed to follow first-order degradation kinetics, and results are presented for both individual hormones and in terms of 17 $\beta$ -estradiol-equivalent or testosterone-equivalent concentrations. Additionally, all modeled lakes were assumed to be closed systems in order to estimate theoretical maximum concentrations in lake water; inclusion of residence time is planned in future versions of the model. Results of the model are presented and compared to reported concentrations, and implications of natural sources of hormones on hormonal regulation in fish species are discussed.

**MP140 Impacts of Sewage Effluents and Ammonia on Reproduction in Zebrafish** G. Van Der Kraak, Univ of Guelph, Dept of Integrative Biology; J. Matsumoto, K. Alkema, Univ of Guelph, Integrative Biology; W. Parker, Univ of Waterloo, Dept of Engineering. We have been studying the effects of treated sewage effluents on the reproductive responses of zebrafish. Effluents subjected to a conventional activated sludge treatment with nitrification (CAS-N) or biological nutrient removal (BNR) including nitrifying and denitrifying conditions and phosphorous removal did not impair spawning success in zebrafish whereas effluent subjected to a conventional activated sludge treatment alone (CAS) led to cessation of egg production. The CAS effluent contained high levels of ammonia (about 6-10 mg/L) which led us to also examine the response of zebrafish to ammonia. In subsequent tests we showed that both CAS effluent or ammonia would block egg production. Moreover, both effluents had no effects on the ovarian expression of steroid acute regulatory protein but markedly reduced the expression of aromatase. In other tests both the CAS effluent and ammonia significantly reduced the hepatic expression of vitellogenin mRNA. Collectively these studies suggest that ammonia may contribute to the reproductive toxicity of sewage effluents and does so by acting at multiple sites along the hypothalamic-pituitary-gonadal axis.

**MP141 Life History Effects of Organic Wastewater Contaminants in the Freshwater Gastropod, *Physa pomilia*** T.O. Luna, Texas Tech Univ/TIEHH, Environmental Toxicology, Texas Tech Univ; C.J. Salice, Texas Tech Univ/TIEHH, Environmental Toxicology, Assistant Professor, Texas Tech Univ. Organic wastewater contaminants (OWCs), including many known endocrine disruptors (EDs) and pharmaceuticals, have been detected in North American surface waters and are cause for toxicological concern. OWCs are not meant to be targeted or applied to the environment, so they haven't been scrutinized or subjected to mandated testing regarding possible adverse environmental effects. OWCs of concern include EDs which have

been shown to exert sublethal effects at environmentally relevant exposure concentrations in a wide range of vertebrate test species. The observed sublethal effects may have effects on the fitness of exposed populations and potential community level consequences. Unfortunately, fewer studies have been aimed at understanding the effects of endocrine disruptors on invertebrates. In many systems invertebrates are important components serving a variety of ecosystem roles. Mollusks, particularly, have strong impacts on primary producers, can be the primary food source for small carnivores and can dominate macroinvertebrate biomass. Our objective was to evaluate the effects of commonly found EDs on life history traits in the freshwater gastropod, *Physa pomilia*. In this experiment two EDs were tested. 17- $\alpha$  ethynyl estradiol (EE2) is a synthetic hormone found in many forms of birth control and 4-nonylphenol (4-NP) is a chemical used in many industrial processes. Snails were exposed to one of the chemicals at one of three concentrations (EE2 – 10  $\mu$ g/L, 1  $\mu$ g/L, 0.1  $\mu$ g/L and 4-NP – 100  $\mu$ g/L, 10  $\mu$ g/L, 1  $\mu$ g/L). Locally found *P. pomilia* juveniles were exposed for two months in a semistatic system with four snails per glass beaker and exposure solutions were changed once per week. We quantified total egg masses laid per beaker per week, total eggs laid per beaker per week, hatching success, and mortality. We found that after two months, snails in the 10  $\mu$ g/L EE2 laid more egg masses than snails in the 1  $\mu$ g/L EE2 ( $p=0.0087$ ) and that snails in the 100  $\mu$ g/L 4-NP laid more eggs per week per beaker than snails in the 10  $\mu$ g/L 4-NP ( $p=0.0298$ ). We concluded from this experiment that there was some evidence that snails in the higher concentrations of EE2 and 4-NP are possibly showing symptoms of superfeminization because of the increased numbers of eggs laid. Future research will include a long term multigenerational study and several behavioral assays to evaluate ecological impacts of EDs that may be mediated through behavioral effects such as predator avoidance.

**MP142 Polybrominated Diphenylether (DE-71) Exposure Alters Reproduction and Endocrine Function in Fathead Minnows (*Pimephales promelas*)** D. Fort, Fort Environmental Laboratories, Fort Environmental Laboratories, Inc.; J. Autry, M. Mathis, C. Fort, H. Fort, B. Todhunter, Fort Environmental Laboratories, Inc.; P. Guiney, SC Johnson & Son. Exposure of *Silurana tropicalis* to DE-71 during development was previously found to increase the proportion of phenotypic females, alter steroidogenesis during sexual differentiation, and induce abnormal ovary development. Previous studies also found that *Xenopus laevis* exposed to DE-71 for 21-d in the EDSP amphibian metamorphosis assay displayed developmental delay resulting from changes in downstream thyroid hormone homeostasis but without direct histological signature in the thyroid glands. To determine if DE-71 altered reproduction and endocrine function in fish, the Tier 1 EDSP 21-day Fish Reproduction Screen with fathead minnow (*Pimephales promelas*) was used. Reproductively-active male and female fish were exposed to DE-71 (0 [control], 0.65, 1.3, 2.5, and 5.0  $\mu$ g/L) via flow-through exposure for 21-d and evaluated for survival, reproductive behaviour, and secondary sexual characteristics. Reproductive fecundity and fertilization success were monitored daily. At termination, the status of the reproductive endocrine system was assessed by the gonad-somatic index (GSI), gonadal histology, plasma steroids (estrogen [E2] and testosterone [T]) and plasma vitellogenin (VTG). Results indicated that the DE-71 exposure did not affect survival or body weight, but reduced male GSI. Reproductive fecundity and gonad histology were altered. Impact on plasma steroid hormone and VTG levels in both sexes will be presented. In general, these results were generally similar to those found in *S. tropicalis* during gonad differentiation and demonstrate the effects of polybrominated diphenylethers on the reproductive system in fathead minnows.

**MP143 Society's Contraceptive Choices and the Resultant Estrogenic Load on the Environment: A US Case Study** U. Khan, McGill Univ; J. Nicell, McGill Univ, Dept of Civil Engineering and Applied Mechanics. More than a decade of evidence suggests that the estrogenic content of wastewaters being released to the environment feminizes fish and that such feminizing effects may potentially comprise the reproductive capacity of the effected fish. Environmentally-relevant estrogens can be broadly categorized in two classes; namely, those that are endogenously produced and those that are exogenously consumed. Of the two, the excretion and therefore the subsequent environmental presence of endogenous estrogens can be considered to be omnipresent since they are a fixed characteristic of our biology. When considering exogenously consumed estrogens used for human contraception, the most important by far in terms of amounts consumed and environmental relevance is the female birth control pill, containing the synthetic



estrogen ethynylestradiol as its active ingredient. The pill was first approved 50-years ago as a form of contraception and is currently used by approximately 11 million women in the USA for this purpose. Since the use of ethynylestradiol, and hence the estrogenic content released as a consequence, is a contraceptive choice made by members of society, a logical question arises: When evaluated from an environmental perspective, should the use of ethynylestradiol be mitigated or even eliminated? And if so, do greener alternatives exist to this contraceptive? How does one go about making such an evaluation? Further, the mere suggestion that society's contraceptive choices be altered to mitigate environmental impact should be analyzed in broader context. Are contraceptive choices that are greener also clinically and personally preferred? Would such a switch to more greener contraceptive choices also be financially feasible? This study will present a first attempt at answering these questions.

**MP144 Sterols in Sewage Treatment Plant Wastewaters and Their Impacts on Salmon** V. Furtula, Environment Canada, Research Scientist; H. Osachoff, Environment Canada, Simon Fraser Univ, Biological Sciences; P. Chambers, Environment Canada, National Water Research Institute; C. Kennedy, Simon Fraser Univ, Dept of Biological Sciences. Fecal material (from sewage treatment plants -STP) contains sterols, which have been recognized as endocrine-disrupting chemicals (EDCs) that can mimic the action of endogenous hormones. The objectives of this study were: (1) to assess the efficiency of STPs for removal of sterols; (2) based on the detected sterol hormones in the effluent, conduct an exposure study of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to environmentally relevant levels. Samples from secondary and tertiary STPs were analyzed by GCMS method for 23 sterols. Fish experiments were conducted that exposed juveniles for 7 days to 5% STP effluents containing estrone and matching estrone-alone levels (2 ng/L). A comparison was made between the estrogenicity of the sewage versus that of the estrone using gene expression changes of four egg protein-related gene transcripts (vitellogenin, vitelline envelope proteins  $\alpha$ ,  $\beta$  and  $\gamma$ ) and four estrogen receptor (ER) gene transcript forms (estrogen receptor  $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$  and  $\beta 2$ ). In the STP influent 14 sterols were detected, cholesterol (459-1061  $\mu\text{g L}^{-1}$ ), coprostanol (393-913  $\mu\text{g L}^{-1}$ ), 24-ethylcoprostanol (145-442  $\mu\text{g L}^{-1}$ ),  $\beta$ -sitosterol (154-415  $\mu\text{g L}^{-1}$ ), coprostanone (110.75-277.04  $\mu\text{g L}^{-1}$ ), campesterol (70.86-176.78  $\mu\text{g L}^{-1}$ ), dihydrocholesterol (33-68  $\mu\text{g L}^{-1}$ ), stigmasterol (26-58  $\mu\text{g L}^{-1}$ ), stigmastanol (26-54  $\mu\text{g L}^{-1}$ ), epicoprostanol (10-25  $\mu\text{g L}^{-1}$ ), desmosterol (5-11  $\mu\text{g L}^{-1}$ ), estriol (10  $\mu\text{g L}^{-1}$ , only detected STP-3), cholestanone (4-8  $\mu\text{g L}^{-1}$ ) and epicholestanol (1-2  $\mu\text{g L}^{-1}$ ). In effluent the same sterols were detected but in much lower concentrations, cholesterol (1-114  $\mu\text{g L}^{-1}$ ), coprostanol (2-99  $\mu\text{g L}^{-1}$ ), 24-ethylcoprostanol (0.6-42  $\mu\text{g L}^{-1}$ ),  $\beta$ -sitosterol (3-37  $\mu\text{g L}^{-1}$ ), stigmasterol (1-20  $\mu\text{g L}^{-1}$ ), coprostanone (0.6-18  $\mu\text{g L}^{-1}$ ), campesterol (1-15  $\mu\text{g L}^{-1}$ ), dihydrocholesterol (0.4-9  $\mu\text{g L}^{-1}$ ), desmosterol (0.1-8  $\mu\text{g L}^{-1}$ ), stigmastanol (0.07-5.  $\mu\text{g L}^{-1}$ ), epicoprostanol (0.13-4  $\mu\text{g L}^{-1}$ ), cholestanone (0.31-3  $\mu\text{g L}^{-1}$ ), epicholestanol (0.07-2  $\mu\text{g L}^{-1}$ ) and estrone (0.03-0.04  $\mu\text{g L}^{-1}$ ). Percentage of sterol removal ranged from 24-100% and varied amongst sterol compounds and with the type of STP. Results of exposure study showed that the ER and egg protein-related transcripts were differentially responsive and somewhat reduced in magnitude in the effluents compared to the estrone-alone. In addition, other endpoints of osmoregulation and vitellogenesis were examined and will be discussed.

**MP145 The Effect of Nitrate, Fe(III) and Humic Acid on the Degradation of Naproxen and Ibuprofen in Photolysis** K. Zoh, Seoul National Univ, Seoul National Univ, Dept of Environmental Health, School of Public Health; J. Im, Y. Kang, Seoul National Univ. Non-steroidal anti-inflammatory drugs, such as naproxen (NPX) and ibuprofen (IBP), are ones of the most detected pharmaceutical compounds in water environment. The previous studies have shown that the sewage wastewater treatment plants (WWTPs) have failed to perfectly remove NPX (0-80%) and IBP (> 81%). Therefore, these compounds can coexist with  $\text{NO}_3^-$ , Fe(III) or humic acid in natural water environment. The aim of this study is to investigate the effect of these coexisting compounds ( $\text{NO}_3^-$ , Fe(III) and humic acid) on the photo-degradation of NPX and IBP in water. We applied single and mixture of environmental factors with various concentrations to NPX and IBP solution under UV-A irradiation. Under photolysis condition with UVA-only (light intensity: 3.5 mW  $\text{cm}^{-2}$ ), the removal efficiency was only 13.7% for NPX and 33.2% for IBP within 180 min, respectively. However, in the presence of 0.47 mg L<sup>-1</sup> for Fe(III) and 20 mg L<sup>-1</sup> for  $\text{NO}_3^-$ , the removal efficiencies of NPX and IBP were enhanced to 49% and 63.1%, respectively.

However, under the same condition in the presence of humic acid (30 mg L<sup>-1</sup>), the removal rates were almost identical with UV-A only reaction. According to these results, Fe(III) and  $\text{NO}_3^-$  ions acted as a main source of OH radical while humic acid acted as a sink of OH radical. This result also implies that the removal of NPX and IBP in photolysis (UVA only) is achieved not only directly by photon but also indirectly by OH radical produced by Fe(III) and  $\text{NO}_3^-$  in water. Our result can help to understand fate of pharmaceuticals in the aquatic system in the presence of sunlight and typical ions and natural organic matters.

**MP146 Triclosan Occurrence in Freshwater Systems in the United States (1999-2010): A Meta-Analysis** A.L. Perez, M. Anderle de Saylor, A. Joseph, M.G. Lew, A. Slocombe, E. Donovan, ChemRisk. Triclosan (TCS) is an antimicrobial substance used in pesticides, hand sanitizers, toothpaste, medical devices, and a number of other consumer products. In recent years, concern has grown regarding the presence of TCS in water due to its potential role in ecological and human health effects, such as microbial resistance and endocrine disruption. In the current investigation, we present a statistical analysis of TCS concentrations in freshwater environments in the United States and quantify exposures to TCS via drinking water. A comprehensive literature review of reported TCS concentrations between 1999 and 2011 was conducted. Data from 41 peer-reviewed and unpublished investigations, including results from 47 states and one US territory was included in the meta-analysis and encompassed the following coded water types: waste, effluent, effluent-impacted environmental, environmental, and finished (n=1500). TCS was most frequently detected in waste waters (93% detection frequency (d.f.), n=214) and concentrations were significantly higher than in effluent waters (82% d.f., n=185). In environmental waters impacted by effluent, TCS concentrations ranged from less than the limit of detection to 1,600 ng/L (58% d.f., n=140), and were significantly greater than TCS measured in non-impacted environmental waters. The upper confidence limit on the mean of all drinking water samples was 19 ng/L (4% d.f., n=172). Although no US regulatory standard exists for TCS in waters, the exposures via drinking water are 12,000 times less than the reported chronic reference dose for dietary exposure of 0.3 mg/kg/day. These results suggest that for the US population, drinking water is not a significant source of TCS exposure. However, the data were suggestive of a seasonal effect for TCS in waters, where concentrations were significantly higher in months with the highest precipitation.

**MP147 The Evaluation of Control Performance in the Fish Short-term Reproduction Assay Against the OPPTS 890.1350 Guideline** L. Sayers, Springborn Smithers Laboratories; R. Biever, US Environmental Protection Agency (USEPA) has begun to execute a new paradigm with the Endocrine Disruptor Screening Program (EDSP). The goal is to prioritize chemicals based on route of exposure and screen them for potential endocrine or thyroid activity. Screening is being conducted using 11 Tier 1 assays. One of those assays is the Fish Short-Term Reproduction Assay. Although the OPPTS 890.1xxx guideline is very specific, it is not a trivial matter to meet the acceptance and performance criteria listed in the assay. Even though this assay has been validated, there are still not enough baseline data to reliably set acceptance or rejection criteria. This presentation will evaluate control performance data from at least 12 assays conducted over the past 10 months and compare this performance against recommended acceptance and performance criteria presented in the OPPTS 890.1350 and OECD 229 guidelines. The pre-exposure endpoints evaluated will include: fecundity expressed as eggs/female/day and number of spawns. The exposure endpoints evaluated will include: survival, fecundity expressed as eggs/female/day, percent fertility, male and female gonad histopathology, gonadal somatic index (GSI) and blood plasma vitellogenin, and male tubercle score. Recommendations regarding acceptance and performance criteria for this guideline will be provided.

**MP149 Effects of a Glucocorticoid Receptor Agonist, Dexamethasone, on Fathead Minnow Reproduction and Development** C. LaLone, USEPA; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; E. Medlock, DePauw Univ, Dept of Biochemistry; M. Kahl, K.M. Jensen, E. Durhan, E. Makynen, USEPA, Mid-Continent Ecology Division; C. Blanksma, R. Johnson, J. Cavallin, S. Seidl, USEPA; L. Thomas, L. Wehmas, USEPA, Mid-Continent Ecology Division; S.Y. Skolness, Univ of Minnesota, Dept of Biochemistry and Molecular Biology; G.T. Ankley, USEPA, Office of Research and Development, National Health and

Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division. Few studies have examined the effects of synthetic glucocorticoids on the reproductive axis of fish, despite the fact that these chemicals are therapeutically prescribed anti-inflammatory agents that are abundantly produced and consumed. To generate data to assess potential risk to the aquatic environment, this study used a fathead minnow 21 day reproduction assay and a 29 day embryo/larvae exposure assay to determine reproductive toxicity and life stage exposure effects to the common human and veterinary drug, dexamethasone (dex). Exposure to 500 µg dex/L caused significant adverse effects on fathead minnow fecundity, female plasma estradiol concentrations, and increased abnormally hatched fry following the 21 d exposure. Female fish exposed to 500 µg dex/L also displayed a significant increase in plasma vitellogenin (vtg) protein levels potentially due to decreased spawning. A decrease in vtg mRNA expression in ovary tissue from females exposed to the high dex concentration lends support to this hypothesis. Concentrations of 0.1 and 50 µg dex/L significantly increased both gonad mass and gonadal somatic indexes for males and females, respectively. Histological results indicate that 29 day embryo/larvae exposure to 500 µg dex/L caused a significant increase in the development of deformed opercula. Taken together these results indicate that non-lethal concentrations of a model glucocorticoid receptor agonist can impair fish reproduction and development. The contents of this abstract neither constitute nor reflect official USEPA policy.

**MP150 Reproductive Impact and Proteomic Analysis of Androgenic and Antiandrogenic Disruptors on the Hermaphroditic Freshwater Gastropod *Lymnaea stagnalis*** A. Giusti, Liege Univ, Laboratory of Animal Ecology et Ecotoxicology; V. Ducrot, INRA, Ecotox & Quality of Aquatic Ecosyst, INRA, Ecotoxicology and Quality of Aquatic Ecosystems; C. Joaquim-Justo, Liege Univ, Laboratory of Animal Ecology et Ecotoxicology; L. Lagadic, INRA, Ecotox & Quality of Aquatic Ecosyst; P. Leprince, Liege Univ, GIGA-Neurosciences; E. DePauw, Liege Univ, Laboratory of Mass Spectrometry; J. Thome, Liege Univ, Laboratory of Animal Ecology et Ecotoxicology. Mechanisms of action of endocrine disruptors on gastropods are poorly understood, and knowledge on their impacts is still scarce in most of species. In this study, effects of two androgens (tributyltin and testosterone), one antiandrogen (cyproterone acetate) and one estrogen (chlordecone) on growth and reproduction were investigated in the hermaphrodite gastropod *Lymnaea stagnalis*. In this study, exposure to a range of concentrations (ng/l to µg/l) of each chemical was performed during 21 days. The number of clutches and the number of eggs per clutch were monitored. A decrease in clutches laid per snail was observed after exposure to tributyltin (540, 1180 and 2600 ng/l) and chlordecone (10, 22, 50 and 110 µg/l). A significant decrease in egg laid per snail was observed after exposure to tributyltin (540, 1180 and 2600 ng/l) and chlordecone (50 and 110 µg/l). An increase of egg abnormalities ratio in exposed snails (atrophied albumen, polyembryonicity,...) was observed in *L. stagnalis* following exposure to testosterone (2, 22, 50 and 110 ng/l), cyproterone acetate (4,5 and 50 µg/l), tributyltin (110 and 244 ng/l) and chlordecone (4,5 and 10 µg/l). Investigation of alteration in protein expression in exposed snails was performed using proteomic analysis such as 2D-DIGE. Mass spectrometry identification was performed on proteins with altered expression. We could establish correlation between reproductive endpoints and changes in proteins involved in egg formation and in egg laying were underlined. Egg yolk ferritin, the main protein of egg yolk, was shown to be reduced significantly in relationship with a decrease of egg yolk quality after exposure to tributyltin 540 ng/l and cyproterone acetate 4,5 µg/l. Ovipostatin, a protein proved to reduce egg masses, was significantly over expressed in snails exposed to 50 µg/l of chlordecone and were in relationship with a reduction of clutches laid by individuals. Further western blot analysis on those proteins involved in the reproduction are underway. These analysis will enable us to confirm and refine with more specificity the 2D-DIGE results for the selected proteins. The results of this study can help to establish new biomarkers of exposure of endocrine disruptors in freshwater environment and can provide new insight on mode of action of endocrine disruptors in *L. stagnalis*.

**MP151 Effects of Diethylstilbestrol in Fathead Minnows: Part 1. Effects on Reproductive Endocrine Function** O. Adediji, Univ of Ibadan, Veterinary Public Health and Preventive Medicine; E. Durhan, M. Kahl, K.M. Jensen, E. Makynen, D.L. Villeneuve, G. Ankley, USEPA, Mid-Continent Ecology Division. Diethylstilbestrol (DES), a synthetic nonsteroidal estrogen, was once widely prescribed to prevent miscarriages, and was used

as a growth promoter in feed for beef and poultry production. After it was determined that DES caused significant adverse effects in the offspring of mothers exposed to the compound, its use was limited in both pharmaceutical and veterinarian applications. However, since DES might still be used as a growth enhancer in aquaculture in some countries, it is important to determine possible endocrine impacts it could have on fish, as well as its potential to accumulate in tissues that might result in exposure of human consumers to DES. A fathead minnow (FHM) experiment was conducted in which adult fish were exposed to DES at 1.0, 10 and 100 ng/L for 96 h followed by a 96 h depuration under flow-through conditions. Several endpoints associated with reproductive endocrine function were evaluated at both time periods. There was a significant concentration dependent decrease in male secondary sex characteristics (tubercle score) at the end of the study. Vitellogenin (Vtg) protein levels were significantly increased in the plasma of male fish exposed to DES at 10 and 100 ng/L. Vitellogenin mRNA and estrogen receptor (esr1) mRNA increased significantly in the male liver in the 10 and 100 ng DES/L exposure groups, while insulin like growth factor (igf1) mRNA showed a significant increase in animals from the 100 ng/L treatment. Overall our results show that that DES is a relatively potent estrogen in fish, capable of disrupting normal endocrine function. The contents of this abstract do not reflect USEPA policy.

**MP152 Effects of Diethylstilbestrol in Fathead Minnows: Part 2. Concentrations in Water and Tissues** E. Durhan, E. Makynen, K.M. Jensen, M. Kahl, USEPA, Mid-Continent Ecology Division; O. Adediji, Univ of Ibadan, Veterinary Public Health and Preventive Medicine; D.L. Villeneuve, G. Ankley, USEPA, Mid-Continent Ecology Division. Diethylstilbestrol (DES), a synthetic nonsteroidal estrogen, was once widely prescribed to prevent miscarriages, and was used as a growth promoter in feed for beef and poultry production. After it was determined that DES caused significant adverse effects in the offspring of mothers exposed to the compound, its use was limited in both pharmaceutical and veterinarian applications. However, since DES might still be used as a growth enhancer in aquaculture in some countries, it is important to determine possible endocrine impacts it could have on fish, as well as its potential to accumulate in tissues that might result in exposure of human consumers to DES. A fathead minnow (FHM) experiment was conducted in which adult fish were exposed to DES at 1.0, 10 and 100 ng/L for 96 h followed by a 96 h depuration period. The direct measurement of DES in tank water and fish tissue samples at ng/L levels proved challenging. The analytical method used was developed on a single quadrupole liquid chromatograph/mass spectrometer (LC/MS). DES was measured on a daily basis using LC/MS with isocratic (tank water) or gradient (fish tissue extract) HPLC elution of a reversed phase column and atmospheric pressure photo-ionization detection. The fish tissue residues were determined by analyzing samples which were extracted using acetonitrile and homogenization techniques. The analytical quantitation limits were 75 ng/L and 2 ng/g for water and tissue samples, respectively. The predicted BCF (bioconcentration factor) of DES based on its log Kow (octanol-water partition coefficient) is 5000, while our experimental BCF was determined to be in the range of 50-100. This suggests a low risk of accumulating substantial amounts of DES in fish tissue. The contents of this abstract do not reflect USEPA policy.

**MP153 Considering Evolution of Life's Response to Potentially Toxic Chemicals** E. Monosson, Independent/UMass. Toxicology has deep evolutionary roots, yet the evolutionary history of how life responds to chemical challenges remains largely unexplored. Even as ecologists, immunologists, medical scientists and others now benefit from more deeply understanding their sciences – toxicologists are just beginning unravel the origins of the toxic response, and apply evolutionary theory to their work. We often consider the adverse human and environmental health impacts caused by chemical toxicants as a modern phenomenon, yet survival of some of the earliest forms of life depended upon the ability to transform, excrete and stow away naturally occurring potentially harmful chemicals. Today, a diversity of species, including humans rely upon these ancient mechanisms which range from highly conserved enzymes like DNA photolyase and metal binding proteins, to p53's role in cancer suppression, integrated defense networks and rapid evolution for protection from both natural and industrial chemical contaminants. This presentation provides specific examples selected from the literature intended to provoke in depth discussion of how and why evolution is relevant to both the basic and applied aspects of toxicology. Would an evolutionary context allow toxicologists and regulators to make

better use of new techniques including 'omics methodologies. Could analysis of chemical mixtures benefit from insights into the networked nature of life's response to chemicals? Might an improved understanding of the evolutionary history and phylogeny of a particular receptor improve predictions of chemical activity across species based on structure activity analysis? How might a greater understanding of contemporary evolution influence assessment of populations inhabiting highly contaminated sites? Considering the most basic origins of the toxic response could add greater depth to a rapidly expanding science, while initiating the growth of new branches on the field's own evolutionary tree.

**MP154 Dioxin-related Compounds in New York House Dust: Chlorinated/Brominated Dioxins/Furans, Dioxin-like PCBs and In Vitro Dioxin-like Activities** N.M. Tue, S. Takahashi, S. Tanabe, Ehime Univ, Center for Marine Environmental Studies; G. Suzuki, National Institute for Environmental Studies; K. Kannan, New York State Dept of Health, Wadsworth Center. Several studies have reported high levels of polybrominated diphenyl ethers (PBDEs) in American house dust as well as human matrices. However, the occurrence and potential exposure risk of polybrominated dibenzo dioxins and furans (PBDD/Fs), which are impurities in PBDE technical mixtures, or products of PBDE thermal/photolytic degradation, in the American home environment have not been investigated. In this study, PBDD/Fs and the legacy dioxin-related compounds (DRCs), polychlorinated dibenzo dioxins/furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) were analyzed in house dust samples collected from New York State. The total dioxin-like activities of persistent (sulfuric acid-resistant) compounds in the samples were also evaluated using the Dioxin-Related Chemical-Activated Luciferase gene expression assay (DR-CALUX). PBDD/Fs, PCDD/Fs and DL-PCBs were detected in the ranges of 0.33-150 (median 2.1), 0.12-80 (median 1.7) and 0.46-35 (median 5.6) ng/g, respectively. Although there were large variations in the concentrations and homolog profiles of DRCs, octa-CDD and hepta-octaBDFs were generally the major homologue groups found in the dust samples. The in vitro dioxin-like activities of the sulfuric acid-treated dust samples were in the range of 30-8000 (median 210) pg BEQ/g, to which PCDD/Fs theoretically contributed < 1%-130%, PBDD/Fs < 1%-21% and DL-PCBs < 1%-6.8% of the total toxicities. With the exception of two samples with very high concentrations of PCDD/Fs (19 and 80 ng/g), a large portion of the DR-CALUX results could not be explained by additive activity of DRCs with known relative potency values (REP). Our results indicate that other persistent compounds in house dust may exert AhR agonist. High levels of dioxin-like activities in American house dust suggest that dust ingestion is a critical pathway for human exposure to DRCs, especially for children.

**MP155 Intensive Indoor Measurements and Biomonitoring of Phthalates: Implications for Exposure** S.R. Chaudhuri, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; M.L. Diamond, Univ of Toronto, Dept of Geography and program in planning, Univ of Toronto, Dept of Geography; E. Mukwede, S. Verkoeyen, Univ of Toronto; J. Brook, Z. Jiang, Environment Canada; A. Wheeler, Health Canada. With North Americans spending 90% of their time indoors, understanding the sources, and fate of semi-volatile organic compounds in the indoor environment is vital. Phthalates (PAE), a diverse group of organic esters, are a ubiquitous class of plasticizers that can be found in personal care items, residential materials, food-contact products, and medical applications. PAE are recognized as endocrine disrupting compounds, and several studies have indicated an association between PAE exposure and allergy and asthma development in children. To determine optimal indoor sampling methods that relate to exposure, an intensive multimedia measurement campaign was conducted in 5 Toronto homes. The study consisted of over 150 simultaneous measurements and sample collections in the bedroom, living room, and kitchen of each home. Measurements included: i) urine based biomonitoring of PAE metabolites in participants, ii) direct indoor home characterization (ex: particulate matter concentration, air-exchange rate), iii) PAE concentration measurements in dust, air, and surface films on windows, and iv) time-activity and product use information through questionnaires and diaries. Dust samples were collected via vacuum, while passive air samplers and window wipes were tested as novel methods for measuring PAE concentrations in indoor air and surface films respectively. Heavier molecular weight di(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP) had the highest concentration in window films in all 5 homes, whereas lighter molecular weight diethyl phthalate (DEP) was found to be the lowest in 4

of the 5 homes. Concentrations ranged from as high as 3 ng/cm<sup>2</sup> of surface area of DEHP to less than 10<sup>-4</sup> ng/cm<sup>2</sup> of DEP. DEHP and DiNP were the most abundant phthalates in all rooms of all homes. Eight urinary PAE metabolites, tested in daily first morning urine samples, varied over an order of magnitude between participants. Investigation of optimal indoor sampling methods will improve the ability to explain fate and exposure of PAE and allow for the suggestion of interventions or preventative measures to minimize exposure to these chemicals.

**MP156 Assessing PAH Residues from "Third Hand" Tobacco Smoke: Significant Vector for Exposure or a Non-Issue?** T. Fleming, Philadelphia Univ, School of Science and Health; C. Cordeiro, L. Quast, Philadelphia Univ; J. Ashley, Philadelphia Univ, Institute for Textile and Apparel Product Safety. Polycyclic aromatic hydrocarbons (PAHs) are a suite of potentially carcinogenic organic compounds generated during the incomplete combustion of carbon-containing material such as tobacco. Though PAH concentrations have been widely studied in first and second hand smoke, there is a paucity of data sets characterizing levels in 'third hand' smoke, the residue adhering to the smoker and smoking environment after combustion. The purpose of this study was to quantify the levels of various PAHs in tobacco smoke residue, or third-hand smoke, and assess whether these concentrations are significant as a vector for exposure to nonsmokers, especially children. To quantify PAH levels within third hand smoke, test subjects' hands used to hold the cigarette were swiped before and after smoking with solvent-saturated cotton gauze. PAHs were subsequently extracted by sonication with hexane. Hand swipes were analyzed for six PAHs (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, and fluoranthene) using gas chromatography mass spectrometry (GC-MS). Smokers' bodies (e.g., hands, apparel) have significantly higher PAH levels than non-smokers. For example, after only one cigarette smoked under ambient conditions, total PAHs were as high as 120 ng/hand for smokers compared to

**MP157 A Potential Protective Effect of Selenium Against Mercury Toxicity in Tropical Coastal Aquatic Species** H.A. Kehrig, Universidade Federal do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; T.G. Seixas, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; C.M. Souza, A.M. Di Benedetto, Universidade Estadual do Norte Fluminense, Laboratório de Ciências Ambientais; O. Malm, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho. Selenium (Se) is nutritionally important as an essential trace-element for all life forms that have nervous systems, but is harmful at slightly higher concentrations. Se reportedly protects mammals against the toxic effects of mercury (Hg). Hg has no known normal metabolic function and its presence in living organisms is potentially hazardous. Hg accumulates in the aquatic food web. It is the chief exposure route for wildlife and humans. The Se:Hg molar ratios approaching or exceeding 1:1 is thought to provide Se-dependent Health Benefits (Se-HB) as protective against Hg toxicity. Evaluation of the health risk posed by Hg exposure from seafood consumption requires concurrent consideration of Se content in the particular species. Se and Hg concentrations, based on a wet weight basis, were evaluated in 531 individuals' muscle tissue of 179 predatory fish (PF), 60 non-predatory fish (NPF), 24 squids (MC), 153 mollusks (MB), 70 shrimps (AC1) and 45 crabs (AC2) from a south-eastern Brazilian coast. These aquatic species are widely distributed in this region and are also often consumed by the human population. All species presented Se in muscle below the maximum allowable selenium concentrations, 2.0 mg.kg<sup>-1</sup>. Only five PF individuals presented Hg in muscle above the maximum permissible limit of 0.50 mg.kg<sup>-1</sup> wet wt. established for human consumption of aquatic species by WHO. As Hg exposure from fish consumption has been the focus of this study, the molar ratio between Se and Hg has been investigated. Se was in molar excess of Hg in almost all species, indicating that substantial Se was available to counter the Hg that was also presented in them. Only 7 individuals presented the Se:Hg of less than 1. Se:Hg might decline with increasing fish length, possibly reducing Se protection in larger fish. The relationship between Se:Hg and total length was poor and significant ( $r = -0.324$ ;  $p < 0.0001$ ). Se-HB value was calculated as Kaneko and Ralston (2007) to better describe and integrate Se-specific nutritional benefits in relation to potential Hg-exposure risks presented by these species evaluated. As a result of their rich Se and low Hg contents, all aquatic species presented Se-HBV higher than 1, suggesting that they have Se to potentially protect them and their consumers against Hg toxicity. *Centropomus*



*undecimalis* (common snook) and *Paralichthys brasiliensis* (banded croaker) had the most favorable Se-HBV.

**MP158 In Vitro Evidence for Biomineralization of the Ternary Hg-Se-S Compound from Inorganic or Methylated Mercury (II)** F. Wang, Univ of Manitoba, Centre for Earth Observation Science, Dept of Environment and Geography; M. Khan, M. Lemes, Univ of Manitoba, Dept of Chemistry. The ultimate metabolic product of biological mercury (Hg) – selenium (Se) antagonism is most likely HgSe(s) which is essentially biochemically inert due to the extremely strong Hg-Se bonding. Although the *in vivo* presence of HgSe(s) granules has been analytically confirmed in the liver and kidneys of numerous species of marine mammals and sea birds, its formation pathway remains unclear. *In vitro* studies from our laboratory revealed that HgSe(s) could be produced in the presence of Se from either inorganic Hg(II) or methylmercury (MeHg) under conditions relevant to the *in vivo* environment. Furthermore, the resulting HgSe(s) is not necessarily present as pure tiemannite but rather in the form of the ternary  $HgSe_xS_{1-x}$  ( $x = 0-1$ ). In the case of inorganic Hg(II), excess amount of glutathione and selenite results in the formation of soluble  $HgSe_xS_{1-x}$  which upon acidification precipitates out and ages into insoluble nanoparticles. In the case of MeHg, both MeHg-thiol and –selenol complexes are found to be kinetically instable, forming  $HgSe_xS_{1-x}$  via the intermediate bis(methylmercuric) sulfide or selenide. The demethylation of MeHg is enhanced by the replacement of thiols with selenols and by an increase in the ionic strength. If confirmed *in vivo*, the presence of the ternary  $HgSe_xS_{1-x}$  product would suggest that Hg detoxification with Se supplement needs to consider the participation of intracellular S which is much more abundant than Se.

**MP159 Interactive Kinetics of Selenomethionine and Methylmercury in Juvenile White Sturgeon (*Acipenser transmontanus*)** S.S. Huang, A.B. Strathe, J.G. Fadel, Univ of California, Davis, Animal Science; P. Lin, National Health Research Institutes, Environmental Health and Occupational Medicine; T. Liu, National Yang Ming Univ, Institute of Environmental and Occupational Health Sciences; S. Hung, Univ of California Davis, Animal Science. Selenium (Se) and mercury (Hg) are prevalent pollutants of industrialized watersheds. However, when co-administered, Se has protective effects on organisms from Hg. The mechanism is not known, but it is thought that Se reduces Hg availability, either by forming biologically inert complexes and/or associating with selenoproteins. Relatively little information on aquatic organisms are available, despite concerns with aquatic contaminations. We hypothesize that the interaction occurs during uptake, deposition and excretion, affecting the fractional rates of these parameters. In the present study, a combined technique of stomach intubation, dorsal aorta cannulation and urinary catheterization was utilized. Selenium and Hg were provided as selenomethionine (SeMet) and methylmercury (MeHg), respectively. Groups of 10 sturgeons were orally intubated with a single dose of either 0 (control), SeMet (500 µg/kg body weight; BW), MeHg (1000 µg/kg BW), or their combination (SeHg; 500 µg Se/kg and 1000 µg MeHg/kg). The Se and Hg content in the sturgeon blood and urine were tracked through-out the 7 time points and at the end of the 6 time periods, respectively. Brain, liver, kidney, a cubical section of the white muscle and the remaining whole body were collected at 48 hr. The preliminary results showed that the sturgeons intubated with SeHg have significantly lower blood Se and Hg levels than in those intubated with either SeMet or MeHg alone. In the SeHg group, Se absorption was completed at 3 hr and level remained near peak at 48 hr, sharing a similar profile as in those given SeMet alone. In contrast, Hg absorption was very rapid, completed by 3 hr, followed by a sharp decline to half of the peak concentration; whereas, in the MeHg only fish, completion of Hg absorption was much slower, at 12 hr, and levels remained elevated. Levels of Se in tissues, particularly in the kidneys, were significantly decreased in the SeHg group than in those given SeMet alone. In contrast, tissue Hg levels in the SeHg were similar to those given MeHg alone, with the exception to the kidneys, where Hg concentration was decreased. Although similar in profile, the cumulative urine excretion of Se was much lower in the SeHg fish than in those given SeMet alone. Low levels of urinary Hg were detected, between 6-12 hr, in the SeHg group, but were not detectable in the MeHg fish. Results of the kinetics analyses and compartmental modeling will be discussed.

**MP160 Abstract not properly submitted** T.A. Jarvis, Valdosta State Univ, Dept of Biology.

**MP161 Technical Challenges Encountered While Conducting the EDSP Fish Short-term Reproduction Assay (FSTRA) and the Amphibian Metamorphosis Assay (AMA)** D. Matlock, C. Banman, T. Alexander, Bayer CropScience; L.S. Ortego, Bayer CropScience, Ecotoxicology, Bayer CropScience. The USEPA has developed a two-tiered system for screening and testing the potential interactions of pesticide and non-pesticide chemicals with the estrogen, androgen and thyroid (EAT) hormonal systems in order to determine if they pose a risk to human health or ecological systems. Assays are currently being conducted to satisfy these requirements, with deadlines due beginning late in 2011. Two of the eleven assays within the tier-1 testing battery are the Fish Short-Term Reproduction Assay (FSTRA) and the Amphibian Metamorphosis Assay (AMA). When conducting these screens, multiple challenges were experienced, including the time, labor and specialized equipment required to conduct the assays. Other challenges included the development of solvent free systems, proper dose selection and the distinction of non-endocrine stressors versus endocrine-mediated effects. Some specific challenges to the FSTRA assay included the variability of reproductive performance common to fathead minnows, unplanned mortality in control or exposure tanks, immature males mistakenly identified as females and insufficient amounts of plasma available for conducting both VTG and sex steroid analysis. Some specific challenges to the AMA included bent spines in developing tadpoles and maintaining adequate development without exceeding stage 60 N&F in control tadpoles. This poster will serve to further discuss these and other challenges encountered while conducting these assays.

**MP162 Importance of Supplemental Sampling During the EDSP Tier 1 Amphibian Metamorphosis Assay – Case Studies** D. Fort, Fort Environmental Laboratories, Fort Environmental Laboratories, Inc.; J. Autry, M. Mathis, Fort Environmental Laboratories, Inc.; S. Pawlowski, BASF, BASF AG. The use of supplemental sampling beyond the general scope of the standard method guidance to help elucidate potentially ambiguous test results will be discussed using two case studies as examples. The current approach to evaluate data from these tests involves individual assessment from primarily morphological data including, stage of development, thyroid gland histology, hind limb length, and measures of growth for the Tier 1 amphibian metamorphosis assay (AMA). Since each of the endpoints is considered individually, a potential for ambiguous or difficult to assess data sets may be produced by these Tier 1 assays. In an effort, the help define potentially ambiguous data sets; collection of additional samples during these assays, such as plasma for thyroid hormones (TH), tail or hind limb tissue samples for TH receptor  $\beta$  (TR $\beta$ ) expression and deiodinase (AMA), and liver for TH metabolism. As examples, studies with the PBDE mixture, DE-71; and the antimicrobial agent, triclosan, will be presented. Exposure to DE-71 altered the developmental rate of *X. laevis* larvae and SVL, but did not alter thyroid pathology or hind limb length. Additional plasma and tissues samples were collected to evaluate effects on TH levels, TR $\beta$  expression, and TH metabolism and transport as downstream mechanisms of determining whether the effects were directly or indirectly on the thyroid axis. Results indicated that larvae exposed to DE-71 had lower plasma T4 and T3 levels and that deiodinase (DI-II) expression was induced. These results demonstrate that the peripheral aspects of the thyroid axis were affected by DE-71 exposure although the primary aspects of thyroid axis inhibition were somewhat ambiguous. Triclosan did not alter the rate of development, but did increase the rate of growth as determined by body weight and snout-vent length. An increase in vascularization of the thyroid gland was potentially confused with hypertrophy requiring further examination of thyroid gland metrics.

**MP163 Response of 2,4-D in the Amphibian Metamorphosis Assay and the Fish Short Term Reproduction Assay** R.J. Currie, The Dow Chemical Company, Toxicology, Environmental Research and Consulting; K. Coady, The Dow Chemical Company, Toxicology & Environmental Research and Consulting, Michigan State Univ, Zoology Dept; T. Marino, J. Thomas, The Dow Chemical Company, Toxicology & Environmental Research and Consulting; V. Kramer, Dow Agrosciences; B. Neal, Exponent; L. Hammond, Industry Task Force II on 2,4-D Research Data. 2,4-dichlorophenoxyacetic acid (2,4-D), a selective postemergence herbicide for the control of broadleaf weeds, was evaluated in both the Amphibian Metamorphosis Assay (AMA) and the Fish Short Term Reproduction Assay (FSTRA). In the AMA, tadpoles were exposed to mean measured 2,4-D concentrations of 0 (water control), 0.273, 3.24, 38.0 and 113 mg/L in a continuous flow-through test system for either 7 or 21 days. In the FSTRA,

fathead minnows were exposed to mean measured 2,4-D concentrations of 0 (water control), 0.245, 3.14, 34.0, and 96.5 mg/L under continuous flow-through conditions for 21 days. These concentrations of 2,4-D were not overtly toxic to either *X. laevis* tadpoles or fathead minnows. In the AMA, there were no signs of advanced development (as measured by developmental stage and hind limb length) or asynchronous development among 2,4-D exposed tadpoles evaluated on either day 7 or day 21 of the exposure, and no significant histopathological effects were observed among thyroid glands from 2,4-D-exposed tadpoles. Therefore, following the AMA decision logic (OPPTS 890.1100 guideline), 2,4-D is considered "likely thyroid inactive" in the AMA. In the FSTRA, there were no significant differences between control and 2,4-D exposed fish in regard to fertility, male and female wet weight and length, male and female gonadal somatic indices, tubercle scores, or blood plasma concentrations of vitellogenin in male and female fish. Furthermore, there were no treatment-related histopathologic changes in the testes or ovaries in any 2,4-D exposed groups. The only significant effect was a decrease in fecundity among fish exposed to 96.5 mg/L 2,4-D. The cause of the reduced fecundity at the highest concentration of 2,4-D tested in the assay was most likely due to a generalized stress response in the fish, rather than due to a specific endocrine mode of action of 2,4-D. Technical issues associated with the in-life conduct and interpretation of the AMA and FSTRA with 2,4-D will be discussed. These studies were sponsored by the Industry Task Force II on 2,4-D research.

**MP164 The Evaluation of Control Performance in the Fish Short-Term Reproduction Assay Against the OPPTS 890.1350 Guideline** D. York, Smithers Viscient; L. Sayers, Springborn Smithers Laboratories; R. Biever, Smithers Viscient, Dept of Environmental Toxicology. The United States Environmental Protection Agency (USEPA) has begun to execute a new paradigm with the Endocrine Disruptor Screening Program (EDSP). The goal is to prioritize chemicals based on route of exposure and screen them for potential endocrine or thyroid activity. Screening is being conducted using 11 Tier 1 assays. One of those assays is the Fish Short-Term Reproduction Assay. Although the OPPTS 890.1350 guideline is very specific, it is not a trivial matter to meet the acceptance and performance criteria listed in the assay. Even though this assay has been validated, there are still not enough baseline data to reliably set acceptance or rejection criteria. This presentation will evaluate control performance data from at least 12 assays conducted over the past 10 months and compare this performance against recommended acceptance and performance criteria presented in the OPPTS 890.1350 and OECD 229 guidelines. The pre-exposure endpoints evaluated will include: fecundity expressed as eggs/female/day and number of spawns. The exposure endpoints evaluated will include: survival, fecundity expressed as eggs/female/day, percent fertility, male and female gonad histopathology, gonadal somatic index (GSI) and blood plasma vitellogenin, and male and female tubercle score. Recommendations regarding acceptance and performance criteria for this guideline will be provided.

**MP165 The Evaluation of Control Performance in the Amphibian Metamorphosis Assay Against the OPPTS 890.1100 Guideline** M. Lee, L. Sayers, Smithers Viscient; R. Biever, Smithers Viscient, Dept of Environmental Toxicology. The United States Environmental Protection Agency (USEPA) has begun to execute a new paradigm with the Endocrine Disruptor Screening Program (EDSP). The goal is to prioritize chemicals based on route of exposure and screen them for potential endocrine or thyroid activity. Screening is being conducted using 11 Tier 1 assays. One of those assays is the Amphibian Metamorphosis Assay. Although the OPPTS 890.1100 guideline is very specific, it is not a trivial matter to meet the acceptance and performance criteria listed in the assay. Even though this assay has been validated, there are still not enough baseline data to reliably set acceptance or rejection criteria. This presentation will evaluate control performance data from at least 8 assays conducted over the past 10 months and compare this performance against recommended acceptance and performance criteria presented in the OPPTS 890.1100 and OECD 231 guidelines. The endpoints evaluated will include: number of day to stage 51; Day 7 developmental stage, hind limb length, snout to vent length and weight; and Day 21 developmental stage, hind limb length, snout to vent length, weight and thyroid histopathology. Recommendations regarding acceptance and performance criteria for this guideline will be provided.

**MP166 The Androgenised Female Stickleback Screen (AFSS) for Detecting Environmental (Anti) Androgens** I. Katsiadaki, Cefas,

Environment and Animal Health. The current OECD test guidelines for screening chemicals with potential endocrine disrupting activity (TG 229 and TG 230) cannot clearly identify androgen antagonists due to the lack of a specific end-point in the 3 core species involved (fathead minnow, zebrafish and medaka). The reported antiandrogenic activity in the aquatic environment based on the Yeast Androgen Screen (YAS), a suitable in vitro test, is substantial. The evidence of high levels of antiandrogens in the environment emphasises the need to develop a suitable assay for chemical screening. The design of the androgenised female stickleback screen (AFSS), currently an OECD guidance document, is highly suited as a screen due to its simplicity and reproducibility in any laboratory. The fish are simultaneously treated with a model androgen (dihydro-testosterone, DHT) at 5<sub>g</sub>/L and a range of concentrations of the putative antiandrogen. Any antiandrogenic activity is detected by the degree of reduction/inhibition of spiggin induction by the DHT treatment. Here we present the results from a retrospective validation of a large dataset using the AFSS produced over a period of 10 years with the participation of four laboratories in Europe. The in vivo exposures included testing of four antiandrogenic compounds using kidney spiggin levels as an end-point. Spiggin was measured by a validated ELISA method. Independent statistical analysis of the dataset revealed that the AFSS can unambiguously and reproducibly detect antiandrogens. All four tested compounds (Flutamide, Fenitrothion, Vinclozolin and Linuron) were characterised as antiandrogens on the basis of the AFSS whilst ammonia (tested as an endocrine negative substance) had no effect. These data were used for validating the AFSS as an OECD guidance document for the detection of EDCs with androgen modulating activity. Key words: antiandrogens, stickleback, validation, OECD guidance document

**MP167 Multi-disciplinary and Multi-scale Climate Change Vulnerability Assessment and Response** T. Wickwire, Exponent; C. Menzie, Exponent, Inc., Exponent, Principal; R. Cantor, Exponent. Though the potential impacts of climate change on the environment remain challenging to pinpoint, there is little doubt that as impacts emerge, those with management response strategies in place will be in the best position to adapt and minimize interruptions to achieving management goals. Drawing on a diverse expertise in ecological management, environmental service assessment, hydrological modeling, climate modeling, human health assessment, infrastructure planning and economic analysis, we propose a multi-tiered strategic approach by which to prepare for the uncertain effects of climate change. The approach incorporates assessments for three broad characteristics of managed facilities including integrity and sustainability of assets and infrastructure, the health and well being of personnel or communities, and the stability of ecological resources. The target of the approach ranges from multi-national businesses to government agencies that operate facilities and land holdings across the US and around the world. The two-tiered approach will yield assessments at (inter)national, regional, and facility scales. Tier 1 provides a national assessment of climate-change vulnerabilities associated with predicted regional changes in climate. Tier 2 is designed to provide detailed assessments at the regional and facility scale. While the approaches are conceptually similar, Tier 2 involves more spatial refinement and facility-specific assessment of activities. Uncertainties related to forecasts and associated implications must be characterized and used as a basis for identifying means to reduce uncertainties and develop probabilistic representations of potential future conditions. Our approach will assist in enhancing long-term planning with respect to climate change vulnerabilities, and improve the review and comparison of design and management alternatives. Additionally, it will provide a framework for application to any type of business or government entity, identifying the key metrics for preparing a national climate change vulnerability map library based on the most current model outputs, improving inter- and intra-facility communication regarding vulnerabilities through the relative risk model, and encouraging prioritization of management of vulnerabilities.

**MP168 Assessing Risk to Salmon from Estrogens in a Water Reuse Project** K. Warner, Golder Associates Inc. One consideration regarding environmental applications for water reuse projects is the presence of estrogenic chemicals in wastewater treatment plant (WWTP) effluent. While the concentrations of natural and synthetic estrogens in surface waters are not currently regulated under state or federal guidelines, reproductive toxicity to aquatic biota from estrogenic compounds has been demonstrated in scientific reports, raising concern about the potential ecological impacts of water reuse projects. The information here presents an ecological risk assessment

performed for a proposed reuse project in Kitsap County, Washington that was designed to deliver reclaimed water from a WWTP to a wetland area and salmon-bearing stream in order to benefit flow in both systems. A hydrologic analysis and review of the current scientific literature were performed to develop an ecological risk assessment modeling the quantities of estrone, estradiol, and ethinyl-estradiol in the WWTP influent and effluent, and the fate of these compounds in the receiving stream. Conservative estimates from the risk assessment and water quality analysis suggest potential salmon exposure to less than 1.5 ng/L of each estrogenic compound modeled, and minimal reproductive toxicity risk to fish after WWTP upgrades and under predicted receiving stream conditions. A discussion of risk assessment considerations for other water quality parameters in reclaimed water projects is included.

**MP169 Development of Models to Predict Pesticide Spray Drift Deposition from Aerial, Airblast and Groundspray Applications** S. Rodney, R.L. Breton, Intrinsik Environmental Sciences, Inc.; T.M. Wolf, Agriculture and Agri-Food Canada; P. Whatling, Cheminova, Inc.; D.R. Moore, Intrinsik Environmental Sciences Inc.; T.L. Estes, J. Hanzas, Stone Environmental Inc. AgDISP is a theoretical, physics-based model used to estimate drift of pesticides from treated fields and to derive buffer widths required to reduce risk to biota. AgDISP is a component of AgDrift and is used to estimate buffer widths. The outputs from the AgDISP model have been shown to overestimate far-field drift, resulting in overly conservative buffer width estimates. We describe an alternative method for estimating drift deposition using models fit to field trial measurements. Drift curves that show the relationship between deposition and downwind distance were developed for aerial, airblast and groundspray application methods. The theoretical constraints of drift deposition were used to select appropriate models to fit to the data (e.g., negative deposition is not possible). The models were tested for goodness-of-fit and the best-fitting models selected. Best-fitting models included two- and three-parameter Weibull and Burr Type III models fit to percent loss versus distance. Selected models were input into Drift BUD v1, an MS Excel-based tool for estimating risk to non-target biota from pesticide spray drift. This tool also calculates buffer sizes for selected protection levels. Drift curves and Drift BUD v1 buffer results were compared with AgDISP/AgDrift estimates.

**MP170 Environmental Risk Assessment of the Intense Sweetener Sucralose** D.B. Huggert, Univ of North Texas, Dept of Biological Sciences; A. Lillicrap, K. Tollefsen, Norwegian Institute for Water Research. Emerging contaminants, such as pharmaceuticals, personal care products and food additives, are receiving considerable attention with respect to their environmental fate and toxicological properties. These contaminants are in constant commercial use and are thus being continually introduced into the environment via wastewater treatment plants (WWTP). This constant exposure scenario suggests that comprehensive environmental fate and ecotoxicological studies are needed to evaluate the environmental risks of emerging contaminants. Sucralose (1,6-dichloro-1,6-dideoxy- $\beta$ -D-fructo-furanosyl 4-chloro-4-deoxy- $\alpha$ -D-galactopyranoside), is an intense sweetener sold under the trade name Splenda<sup>TM</sup>. The majority of orally ingested sucralose is excreted as unchanged parent compound, with < 1% of the original oral dose excreted as two glucuronide adduct metabolites. Once excreted, sucralose is not readily degraded within the wastewater treatment process and has been detected in municipal effluents and surface waters. Sucralose bioconcentrations factors are < 2.2 in algae, crustaceans and fish, indicating a low potential to bioaccumulate in the food chain. A battery of acute (rainbow trout, bluegill sunfish, *Daphnia magna*, green algae) and chronic (mysid shrimp, *Daphnia magna*, *Lemna gibba*) ecotoxicological studies have been conducted to evaluate the potential of sucralose to elicit toxicity on aquatic organisms. Sucralose concentrations  $\geq 93$  mg/L did not elicit changes in survival, growth or reproduction in any of the standard testing organisms. A wide margin of safety exists when ecotoxicological data are compared to predicted or measured aqueous sucralose concentrations, indicating that sucralose presents a low risk to the environment.

**MP171 Estimating the Potential Significance of Dermal Exposure of Birds, Reptiles, Amphibians, and Mammals to Pesticides** T. Crk, E. Odenkirchen, US Environmental Protection Agency, Office of Pesticide Programs, Environmental Fate and Effects Division; T. Purucker, US Environmental Protection Agency, Office of Research and Development; D. Spatz, N. Mastrota, K. Garber, J. Housenger, F. Farruggia, M. Wagman,

M. Clock-Rust, V. Woodard, T. Downen, H. Yingling, US Environmental Protection Agency, Office of Pesticide Programs, Environmental Fate and Effects Division. The Dermal Uptake Screening Tool (DUST v. 1.0) is a model developed by the United States Environmental Protection Agency's Office of Pesticide Programs. The output of the model will be used in the problem formulation phase of ecological risk assessment to assess the potential significance of chemical exposure to the dermis of birds, reptiles, amphibians, and mammals as a result of pesticide application. Exposure estimates are based on pesticide application rate, application method, and biology of the non-target organism. DUST evaluates dermal exposures via contact with soil and foliar residues and interception of spray. Soil exposure estimates for terrestrial wildlife account for contact with soil particulates via dust bathing and treading the soil surface. Dermal contact estimates vary across terrestrial taxa more than for other exposure pathways, for example, amphibian dermal contact from enhanced skin permeability can be a key exposure pathway due to high dermis-soil water uptake rates that allow amphibians to regulate hydration. DUST output is used to establish a rationale in problem formulation to either dismiss dermal exposure concerns for soil residues and other dermal contact routes or support further consideration in data collection and analysis phases of the ecological risk assessment. This poster includes the following elements of the model: exposure estimation, preliminary effects endpoint estimation, integration of exposure and effects, decision criteria, model assumptions, and model evaluation.

**MP172 Guidance for a Weight-of-Evidence Approach to Ecological Risk Assessments in British Columbia** M.E. McArdle, Exponent, Inc.; A. Fairbrother, Exponent, EcoSciences, Exponent, Inc., EcoSciences; S. Kane Driscoll, Exponent Inc.; C. Menzie, Exponent, Inc., Exponent, Principal. This poster presents guidance for developing a weight-of-evidence (WOE) approach for conducting detailed ecological risk assessments (DERAs) in British Columbia. While WOE has many definitions, it is defined here as the process by which measurement endpoints, which are closely linked to lines of evidence (LOEs), are integrated to evaluate the likelihood and magnitude of ecological risk for an assessment endpoint. This guidance was written to help risk assessors develop WOE assessments that are objective, transparent, and scientifically rigorous. The approach presented in the guidance is anticipated to be the default WOE approach for conducting DERAs in British Columbia; however, alternative approaches may be employed in conjunction with a clear and defensible communication of the structure and merits of the alternative. The guidance describes the use of WOE to select LOEs and assign weighting factors that reflect the quality and relevance of each LOE to its corresponding assessment endpoint. During the risk characterization stage, the weight given to each LOE is revisited and adjusted to account for any unforeseen events during the collection or analysis of a sample that affected the quality or quantity of data, the appropriateness of an established data analysis method, and/or the sensitivity or representativeness of the LOE. The risk assessor considers the magnitude of response for each LOE, together with the weight of each LOE, to reach a conclusion regarding risk. Examples are provided at key steps in the WOE process.

**MP173 Indicators of Ecological Integrity evaluated in Tecolutla, Veracruz, Mexico** P. Ramirez Romero, Universidad Autonoma Metropolitana, Hidrobiologia, U.A.M. Iztapalapa, Depto. De Hidrobiologia; X. Guzman Garcia, G. Barrera Escorcia, S. Gonzalez Rebollar, L. Elizalde Ramirez, Universidad Autonoma Metropolitana, Hidrobiologia. The development of environmental monitoring programs in Mexico is scarce. Mexican standards demand the assessment of recognized indicators in environmental monitoring, including analysis of water sanitary quality by fecal coliform bacteria and chemical contaminants such as metals. Currently we are developing a research project at the Metropolitan Autonomous Univ to generate data on several indicators of ecological integrity in aquatic environments in Mexico, which began in Tecolutla estuary, Veracruz. We generated data on physico-chemical parameters of the estuary, including some contaminants such as coliform bacteria, metals (Cr and Cu), and the toxicity of water and sediments of five collection sites. Bioassays were conducted with *Artemia salina* in water samples and with *Daphnia magna* with sediments after extraction of contaminants by sonication. So far, the analysis indicates poor sanitary quality in two locations, and the concentrations of Cr and Cu are right in line, or above the accepted limits in water, for all locations. The LC<sub>50</sub> with 95% confidence intervals did not allow detection of water toxicity, but in the sediment, it fluctuated between 29.82 % and 41.41% in the first three



sites, and 15.65 % and 13.01% at stations 4 and 5, respectively. This first approach indicates a strong influence of human activities on the estuary.

**MP174 Reduction of PCBs in Striped Bass (*Morone saxatilis*) from Chesapeake Bay Through Adipose Tissue Removal** B. wang, Univ of Maryland, Baltimore County (UMBC), Civil and Environmental Engineering; P. Paul, U. Ghosh, Univ of Maryland, Baltimore County (UMBC). The work presented is an effort to assess human health risks associated with carcinogenic chemicals found in fish caught in Maryland. The Maryland Dept of the Environment (MDE) collects fish samples from Maryland waters to evaluate human health risks from toxic chemical accumulation. Polychlorinated biphenyls (PCBs), a class of anthropogenic chemicals, are the major risk drivers for fish species in the Chesapeake Bay and its tributaries. The PCB concentrations in fish tissues are compared across geographical regions and to Maryland fish consumption advisory levels. In this study we analyzed striped bass (*Morone saxatilis*) samples, a popular gamefish from the Bay in Maryland, collected during fall 2009 and spring 2010 for the measurement of total PCBs and to study the impact of fat trimming on the PCB concentration in the fish tissue. Fillets of the samples were prepared in two different ways- with skin and ribs on and with skin and ribs removed. A total of 34 samples were analyzed. The measurement shows a significant decline, more than half, in the level of PCBs in striped bass compared to analysis from 2001 to 2005. It means the recommended meal limits for the general population for smaller striped bass caught in the Chesapeake Bay increased by 50 percent. Meanwhile, differences were found between PCB concentrations and lipid contents in different prepared samples. Removal of skin and ribs reduces an average of PCB and lipid 84% and 75% respectively for the 2009 fish samples and 88% and 92% respectively for the 2010 fish samples. A strong correlation ( $r^2 = 0.8$ ) was observed between PCB concentration and lipid percent in the whole fillet tissues from fall 2009. For spring 2010 samples a weak correlation ( $r^2 = 0.2$ ) was observed, which could be attributed to spawning which occurs during spring. The correlation verifies that lipid content has the direct effect of increasing accumulation of PCBs in fish tissues. To minimize exposure to lipophilic pollutants, Maryland fish consumption advisories also provide the general guidelines for healthy fish consumption. For example, it is suggested to cut off the fat or use cooking methods such as baking, grilling, or broiling to drip away fat.

**MP175 Risks of Carbofuran Formulations to Birds in Colombia** D.R. Moore, Intrinsik Environmental Sciences (US), Inc; K.J. Beckett, Stantec Consulting Services, Senior Scientist; S. Teed, Intrinsik Environmental Science Inc., Intrinsik Environment Sciences Inc. Carbofuran is a contact and systemic insecticide, nematicide, and miticide belonging to the carbamate class of cholinesterase-inhibiting chemicals. Flowable and granular formulations of carbofuran are commercialized in Colombia. For the flowable formulation, we undertook a refined avian assessment using a recently developed model, the Liquid Pesticide Avian Risk Assessment Model (LiquidPARAM). The focus of the flowable formulation assessment was on one or two banded or in-furrow applications to potato fields in Colombia because this use pattern is the most widespread and has the highest application rate. For each of nine bird species assessed for potato fields in Colombia, LiquidPARAM estimated the maximum retained dose (i.e., maximum body residue level) that occurred over a period of 28 days following application of the flowable formulation in each of 20 birds on each of 1000 potato fields in Colombia. The model has a one-hour time step. For each bird, the maximum retained dose was compared to a randomly chosen value from the appropriate dose-response curve to determine whether the bird survived (maximum retained dose < randomly chosen value from dose-response curve) or died. Collectively, the modeling line of evidence indicated that the community of birds foraging in treated potato fields in Colombia would have greater than 99% survival for both banded and in-furrow application of the flowable formulation. The focus of the assessment for the granular formulation was a single banded or in-furrow application of the granular formulation to potato fields because this use pattern is the most widespread in Colombia. This assessment relied on an advanced probabilistic risk model for birds called the Granular Pesticide Avian Risk Assessment Model (GranPARAM). The modeling line of evidence indicated that application of granular carbofuran would result in 99% and 98% of the bird community surviving following in-furrow and banded applications, respectively. However, highly sensitive, small passerine species with high grit intake rates are predicted to experience 28% mortality following banded application. No bird species experienced high mortality with in-furrow application of

granular formulation to potato fields in Colombia. Field studies conducted in the United States corroborate the predictions of LiquidPARAM and GranPARAM. The assessment indicates acceptable risks of flowable and granular formulations to birds in Colombia.

**MP176 The Use of Pesticide Ecological Incident Data in the US Environmental Protection Agency's Pesticide Ecological Risk Assessments** M. Panger, USEPA, Environmental Fate and Effects Division. The US Environmental Protection Agency (USEPA) uses data on ecological incidents (information on adverse effects to the environment from pesticide use) in its pesticide ecological risk assessments. Incident data can provide evidence that the risk predictions from screening level assessments are supported by actual effects in the field. Additionally, pesticide incidents can help raise awareness of potential risk issues not previously predicted by other standard assessment methodologies. The EPA relies on three primary sources of ecological incident data: the Ecological Incident Information System (EIIIS); the Incident Data System (IDS) (for aggregate reports); and the American Bird Conservancy's Avian Incident Monitoring System (AIMS) (specifically for pesticide incidents involving birds). Information in these databases comes from a variety of sources, including pesticide companies (the 'registrants'), government agencies (both federal and state), public interest groups, and others (e.g., the National Pesticide Information Center). Registrants have reporting requirements for pesticide ecological incidents under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); however, reporting of incidents from non-registrants is voluntary. The limitations and benefits of each database will be discussed along with how incident information is specifically used in EPA's pesticide ecological risk assessments.

**MP177 TVA Kingston Ash Spill: A Preliminary Sediment Triad Analysis for Metals and Legacy Contaminants in the Clinch River** A.R. Stojak, ARCADIS; T.F. Baker, Tennessee Valley Authority; R.M. Sherrard, Tennessee Valley Authority, Kingston Ash Recovery Project; J.G. Smith, ORNL, UT-Battelle, Environmental Sciences Division; J. Iannuzzi, D. Jones, S. Young, ARCADIS; N.E. Carriker, TVA Kingston Ash Recovery Project, Kingston Fly Ash Recovery Project. The 2008 Kingston Fossil Plant ash spill discharged fly ash into the Emory River, with subsequent migration of part of the ash into the Clinch and Tennessee Rivers. The river system sampling and analysis plan includes investigations of sediment, ash, porewater, and surface water chemistries, and potential effects on benthic invertebrates, fish, and wildlife receptors. A sediment quality triad analysis was performed as part of the baseline ecological risk assessment. Sediment chemistry, sediment toxicity, and benthic invertebrate community data formed the three primary lines of evidence. Analyses were completed first for the Clinch River, which is the focus of the triad analysis outlined below. Physical and chemical analysis focused on ash, metals, and metalloids, but included legacy constituents (PCBs, pesticides, and radionuclides) for a subset of samples. Clinch River results showed a spatial distribution of percent ash content and, to varying degrees, metals and metalloids. Laboratory toxicity tests included *Ceriodaphnia dubia* 3-brood survival and reproduction tests; and *Hyalella azteca* and *Chironomus dilutus* 10-day and long-term survival and growth tests. Clinch River screening tests indicated: no significant reductions in survival; significant reductions in growth of *C. dilutus* in 2 of 8 samples; and significant reductions in growth of *H. azteca* in 3 of 8 samples. The definitive tests showed significant effects for survival and emergence (*C. dilutus*) and growth (*H. azteca*) in CRM 1.5, and growth (*H. azteca*) in CRM 4.5. Benthic invertebrate community surveys were performed at fixed transect locations approximately annually. Community composition varied among Clinch River sites, but appeared due primarily to variations in habitat rather than proximity to the release. The sediment quality triad analysis systematically weighed and integrated the lines of evidence to characterize potential risks and identify likely causes. This included qualitative and multivariate analysis of sediment and porewater chemistries, laboratory toxicity responses, community composition, and habitat characteristics. This poster presents an overview of the sediment triad approach and preliminary findings for the Clinch River system.

**MP178 Use of Best Available Data in a Pacific Salmonid Endangered Species Assessment for Prometryn** J. Overmyer, Syngenta Crop Protection, LLC, Ecological Sciences, Americas; J. Bang, Syngenta Crop Protection, LLC, Environmental Safety; K. Henry, Syngenta Crop Protection, LLC, Ecological Sciences; K. Kabler, J. Perine, D. Perkins, N. Peranginangin, S. Pyles, Syngenta Crop Protection, LLC; S.B. Wall,

Syngenta Crop Protection, LLC., Ecological Sciences. Prometryn is an s-triazine herbicide used to control annual broadleaf weeds and grasses in cotton, celery, carrots and other vegetable crops. A biological opinion (BiOp) for the use of prometryn near Pacific salmonid habitats in the states of California, Oregon, Washington and Idaho is scheduled for development by the National Marine Fisheries Service (NMFS). In regard to the pending BiOp, Syngenta is working to identify the best available data for determining the potential exposure/effects of prometryn uses near salmonid habitats in these states. Information to be used in the risk assessment includes data from guideline environmental fate and toxicity tests and other sources. Geospatial approaches used to identify areas of overlap between salmonid habitat and crops with prometryn use as well as to evaluate vegetation within riparian areas near historical prometryn use areas will be presented. In addition, watershed modeling will be presented to contextualize field monitoring residues. The use of these data and other pertinent information for evaluating the potential exposure and effects of prometryn on Pacific salmonids will be discussed.

**MP179 Using Scientific Principles to Expedite Site Closure** J. Gravenmier, A. Francisco, J. Nedoff, E. Kalve, ARCADIS. When evaluating risks to ecological receptors from exposure to contaminated sediments, uptake and accumulation of constituents through the food web is often a critical exposure pathway. Food web exposures are controlled by various physico-chemical factors internal and external to the ecosystem (climate, seasonality, sediment and water quality conditions, habitat types/conditions, species composition, carbon sources/types/quality, etc.). These factors control the bioaccessibility and bioavailability of chemicals in the sediments and the system. Methods for assessing bioavailability of chemicals in sediments on a site-specific basis are inconsistent, and too often risk evaluations are based on either generic information (such as literature-based screening levels or other thresholds) or the total concentration of a chemical(s) in sediments. This can result in an overestimation of risk for particular chemicals, and perhaps leading to unnecessary remediation. A California intertidal salt marsh that has had a variety of historical industrial and municipal inputs provides a good case study for using scientific principles to expedite site closure. The marsh has been investigated for many years under a voluntary program to assess the sediment, surface water, and habitat quality. Although useful data were collected, the investigation program lacked a scientific basis and little progress was made toward site closure. Using a two-tiered screening process, we were able to first conservatively identify site-specific constituents of potential concern (COPCs) in sediment and surface water, and then narrow the list of COPCs through an evaluation of the available site-specific toxicity test data. The combination of the tiered screening evaluation and the development and use of site-specific conceptual site models (CSMs) for the identified receptors provides a scientific basis to assess the additional site data needs, appropriately identify potential risks from COPC exposure, and evaluate if remedial actions are necessary to mitigate those risks.

**MP180 Whole Body to Liver Ratio of Dioxins, Furans, and PCBs in Small Mammals from the Tittabawassee River Floodplain** Y.B. Atalay, Cardno Entrix, Civil and Environmental Engineering Dept; D. Kay, M. Johnstone, P. Bradley, Cardno – ENTRIX; M.J. Zwiernik, Michigan State Univ, Dept of Animal Science. Shrews and voles were collected from the Tittabawassee River floodplain and analyzed for dioxins, furans, and PCBs for ecological risk assessment purposes. Specifically, these small mammals were collected, and whole bodies were analyzed to support the dietary exposure assessment of apical predators. The liver was removed from a subset of the small mammals and analyzed separately from the liverless body. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are persistent contaminants that occur throughout the environment as a result of anthropogenic activities and bioaccumulation. These compounds activate the aryl hydrocarbon receptor signal transduction pathway which involves the induction of cytochrome P450 enzymes including CYP1A1 and CYP1A2. CYP1A2 is highly expressed in the liver and is thought to function in hepatic sequestration. Consequently, much of the toxicity data available in the literature is based on chemical burdens in the liver rather than the whole body. Therefore, while whole body analysis of small mammals provides valuable information for dietary risk assessment of higher trophic levels it has limited value for considering risk to the small mammals themselves. The paired liver and liverless body analysis for 11 small mammals, including two shrew species and meadow vole, was conducted to establish the relationship between liver and whole body

concentrations. There is a linear relationship for lipid normalized concentrations of dioxins, furans and PCBs in liver and whole body with an  $R^2$  of  $>0.9$  for masked shrew, short tailed shrew, and meadow vole. Dioxin, furan, and PCB congener-specific liver to whole body ratios generally range from 1 to 15. Overall, ratios are lower for meadow voles compared to shrews. These ratios can be applied to extrapolate from whole body to liver concentrations to facilitate risk assessment by allowing comparisons to existing small mammal toxicity laboratory studies.

**MP181 A New Rapid Test to Estimate the Influence of Chemical Substance on Algal Growth Based on Delayed Fluorescence** M. Katsumata, Hamamatsu Photonics K.K., Central Research Laboratory; T. Koike, Hamamatsu Photonics K. K., Electron Tube Center; K. Kazumura, A. Takeuchi, Y. Kobayashi, Y. Sato, Hamamatsu Photonics K. K., Central Research Laboratory; N. Tatarazako, National Institute for Environmental Studies, Center for Environmental Risk Research, National Institute for Environmental Studies, Endocrine Disrupter Research Laboratory; Y. Sugaya, National Institute for Environmental Studies, Research Center for Environmental Risk. We are investigating the utility of delayed fluorescence (DF) from algae as a rapid and simple measurement method to estimate algal growth inhibition. The DF is a special type of luminescence; it detects the growth of only those cells that have photosynthetic capability. Since the DF originates from re-excitation of chlorophyll by a reverse reaction of photosynthetic electron transfer, the DF inhibition reflects inhibition of photosynthetic activity that is necessary for growth. Therefore the DF is a potential endpoint for estimation of the influence of chemical substance of algal growth in a shorter time than the conventional 72 hours growth inhibition test (e.g., OECD test guideline 201, TG201). We have developed a rapid and simple test protocol for the DF inhibition test using an ultra-sensitive luminometer that measures the DF in a simple operation. The test protocol uses the green alga *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*). In our protocol, the algae is prepared as a frozen ( $-80^{\circ}\text{C}$ ) sample. The test algae are prepared by thawing the frozen algae 1 hour prior to the start of the experiment in order that the cultured cells are in the exponential growth phase. The prepared test algae can be immediately exposed to a test chemical substance in small culture tubes (volume is 10 ml). The DF from algae in the culture tube is directly measured by the ultra-sensitive luminometer at 15 minutes, 1 hour, 4 hours, 8 hours and 24 hours after exposing of the sample. The culture tube does not need to be opened. The DF is inhibited by the influence of the test chemical substance. From the relationship between the exposure concentration and the DF inhibition, the median effective concentration ( $\text{EC}_{50}$ ) is calculated. Our results of 40 substances for which the  $\text{EC}_{50}$  values are already known (9 phenols, 8 anilines, 6 amines, 9 carboxylic acids, 3 alcohols and 5 others) measured at 15 minutes to 24 hours after the exposure have good correlation with the  $\text{EC}_{50}$  values obtained according to the TG201 standard method. In addition, even after 15 minutes, there is good correlation.

**MP182 An Analysis of T-REX for Use in Assessing Pesticide Risk to Threatened and Endangered Birds** J. Sullivan, Ardea Consulting; J.D. Wisk, BASF Corporation, Ecotoxicology. The US Environmental Protection Agency developed and uses the Terrestrial Residue Exposure (T-REX) model to estimate pesticide residues on wildlife food. The specific food categories included are tall and short grass, broad-leaved plants, seeds, fruits, pods, and large and small insects. Three bird body sizes (20 g, 100 g, and 1000 g) are included in the standard T-REX output. The US Fish and Wildlife Service lists 93 bird species, subspecies, or populations as threatened or endangered (T&E). We assessed whether T-REX adequately estimates pesticide exposures for T&E birds. The avian food-body size combination with the highest estimated residues is short grass and 20 g birds. We discovered that no T&E bird weighing approximately 20 g that eats short grass exists. Many listed species are represented when T-REX considers risk for listed insectivorous species in the 20 g size class. However, no species in the larger size classes of 100 and 1000 g are predominantly insectivorous. Fruit and seed eating listed species occur in each of the size classes that T-REX uses, so the T-REX analysis will appropriately represent the potential risk for fruit or seed-eating listed birds. T-REX does not include any EECs for aquatic dietary items or nectar, terrestrial vertebrates and non-insect invertebrates. Roughly one-third of the listed species have diets that do not emphasize any of the dietary items included in T-REX. Slightly more than half the species have diets that include large contributions by dietary items not included in T-REX.

**MP183 Application of LiDAR-derived High-resolution Elevation Data for Characterizing Natural and Agricultural Features Affecting the Transport of Pesticides**

J. Amos, Waterborne Environmental, Inc., GIS Specialist, Waterborne Environmental, Incorporated, GIS Specialist; C.M. Holmes, Waterborne Environmental, Inc.; J. Bang, Syngenta Crop Protection, LLC, Environmental Safety; L. Fish, Syngenta Crop Protection, LLC, Environmental Safety; P. Hendley, Syngenta Crop Protection, LLC., Senior Syngenta Fellow. Terrain elevation data play an important role in characterizing natural and anthropogenic agricultural features affecting the transport of water, sediment, nutrient, and chemicals at the field and watershed scale. LiDAR (Light Detection And Ranging) is emerging as a technology of choice due to their high resolution and accuracy and increasing public availability. This study was conducted to examine the utility of a LiDAR -derived DEM (digital elevation model) in assessing natural and managed agricultural features potentially affecting runoff covering a 60 square mile region in Nebraska. Processing was conducted using publically available LiDAR and Geographic Information System (GIS) data within standard GIS software and functions. Quantitative metrics describing the topography, hydrology, and natural vegetation elevation were calculated from the LiDAR data and summarized for all fields in the study area. Integrating high resolution aerial imagery with the LiDAR data enabled the identification of engineered features such as terracing, overhead irrigation, and intakes for tile terracing systems. Natural processes identified in the data include concentrated and diffuse flow patterns towards the edge of a field; in-field depressions with the potential for surface water storage; and vegetation diversity of riparian areas characterized by canopy height and density. This study is intended as an initial examination into the utility of LiDAR to assess field runoff vulnerability and assist with stewardship and best management practices.

**MP184 Application of the SWAT Model to Assess California Red-legged Frog Exposure to an Insecticide with Agricultural and Residential Uses**

M. Winchell, Stone Environmental, Inc., Senior GIS Specialist, Stone Environmental, Inc., Senior GIS Specialist, Hydrologist; S. Folle, Stone Environmental, Inc.; R.L. Breton, Intrinsik Environmental Sciences, Inc.; P. Whatling, Cheminova, Inc., Senior Manager of Regulatory Science. The estimation of environmental concentrations of pesticides for use in quantifying the risk of exposure to endangered species for regulatory purposes is based upon a single field model (PRZM) draining into a static pond model (EXAMS). While this approach provides conservative estimations of environmental concentrations (EECs) that could potentially impact species such as the California Red-Legged Frog (CRLF), it does not represent the concentrations that would be expected to occur in habitat areas that are influenced by more complex hydrology and heterogeneous land use/agronomic conditions. To evaluate pesticide concentrations in these more complex aquatic habitats, a Soil and Water Assessment Tool (SWAT) model was developed for a highly vulnerable watershed of significant importance to CRLF habitat. Several candidate watersheds were identified through a spatial analysis exercise that assessed cumulative upstream pesticide use in each NHDPlus stream segment flowing through CRLF core and critical habitat areas. The soil characteristics of the watersheds containing stream segments with the highest pesticide use intensity were assessed and the watershed with the greatest proportion of vulnerable soils was selected. The SWAT model was constructed to allow a wide range of pesticide application scenarios based upon historical use patterns extracted from the California PUR database. Given the uncertainty of the spatial and temporal distribution of pesticide applications at the watershed scale, a Monte Carlo approach was adopted to simulate a large number of possible 30-year pesticide application scenarios. This allowed for the prediction of a more complete range of possible pesticide EECs to which the CRLF may be exposed. The approach developed expands upon standard field-scale modeling methods, resulting in a more realistic simulation of complex environments important to ecological risk assessments.

**MP185 Are Measures of Biochemical Parameters Reliable Predictors of Long-term Ecologically Significant Effects?**

K. Jenkins, S. Brenda, S. Huntley, J. Holder, P. Goodrum, ERM. Evaluations of risk/injuries to ecological receptors have most often focused on measures of mortality, impaired growth and reproduction. These measures of injury are easily understood and provide information on both acute and chronic toxicity. These data can also be incorporated into quantitative population models that can be used to evaluate the effects of increased mortality or reduced reproduction on the sustainability of local populations. Field studies evaluating metrics such as

species abundance and diversity have also been used to directly measure the condition of populations or ecological communities in the field. However, field studies are challenging for very large sites such as that being evaluated following the Deepwater Horizon Oil Spill. Further it is often difficult to relate changes at the population/community level to specific events. Recently, it has been suggested that biochemical parameters used to evaluate chemical exposure history as well as possible mechanisms of toxicity, can also be used as measures of possible ecologically significant effects. For example in response to an oil spill, the cytochrome P450 1A (CYP1A) enzyme, can be induced in response to exposure to certain petroleum related compounds is often used as an indicator of exposure. It has also been proposed as a measure of deleterious effects. However, in the absence of a comprehensive evaluation of the relationship between biochemical responses and overt toxic responses, the reliability of biochemical parameters such as CYP1A to predict overt toxicity to individuals, is equivocal at best. Such an evaluation must consider, at a minimum: (1) the specificity of the biochemical parameter; (2) the mode of action for the toxic response of interest; (3) the implications of various intervening and confounding variables; and (4) the degree of parallelism in dose-response between the biochemical parameter and the toxic response or higher level service losses. Based on an evaluation of the available data for several biomarkers including CYP1A in fish we conclude that while some biochemical parameters may provide indirect information regarding exposure to classes of chemicals, they are not reliable predictors of ecologically significant risk or injury to individuals or populations. The basis for these conclusions will be discussed.

**MP186 Comparison of Three Leaching Models of Different Complexity for Estimating Shallow Groundwater Concentrations**

T.L. Estes, Stone Environmental, Inc.; W. Chen, Syngenta Crop Protection LLC. Leaching of agricultural chemicals in soil depends on a number of factors, including field hydrological properties, local meteorology, use patterns and environmental fate characteristics of the agricultural chemical. Computer models for chemical soil leaching assessment take into account these factors to a varying degree. This presentation evaluates three models, SCI-GROW, PRZM, and WINPRZM, each of which has a different level of representation for these factors. SCI-GROW is a Tier 1 US Environmental Protection Agency (EPA) regulatory model which is based on a regression calibrated with shallow groundwater monitoring data. PRZM, also an EPA regulatory model, is a comprehensive field scale model which takes into account all major hydrological, meteorological, and environmental fate factors in an agricultural field. WINPRZM is a European regulatory model which was developed based on PRZM with an enhanced environmental fate subroutine incorporating time-dependent sorption. For each of the models, a regional scale soil data set is used and distributions of model outputs will be compared using a group of statistical metrics to evaluate the sensitivity of the processes each model represents. Propagation of variability in inputs and simplification of the environmental fate processes as well as the validity of using simple models for regional scale leaching assessments will be examined.

**MP187 Conceptual Model for Ecological Risk Assessment in the Aquatic Environment Using Environmental Monitoring Programs**

K. Tallini, Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus, Biotechnology; R.d. Magali, Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus, Environmental; M.T. Raya-Rodrigues, Federal Univ of Rio Grande do Sul, Ecology Center. The aim of this study was to establish a conceptual model for ecological risk assessment for the aquatic environment using data from environmental monitoring programs for surface water and sediment compartments, usually established by environmental monitoring agencies in Brazil. The investigative scenario was based on chemical, ecotoxicological and biological data from environmental monitoring in Baixo Jacuí, in the town of São Jerônimo, in the State of Rio Grande do Sul, Brazil. The proposed model for ecological risk assessment in aquatic environments is composed of four stages. The first stage of the risk assessment model entails collecting chemical, ecotoxicological and biological information on surface water and sediments in four different sampling locations of the environment under study. The second stage involves investigating the chemical and ecotoxicological evidence on the basis of applying Brazilian environmental legislation to the Resolutions of the National Environmental Council No. 357/2005 for surface water and No. 344/2004 for sediments. In this model, the lines of evidence registered in the abiotic compartments were treated as an explanatory variable. The chemical evidence resulted from the presence of



Hg, Pb and Zn and the ecotoxicological evidence from the aquatic toxicity tests with *Ceriodaphnia dubia* for surface water and *Hyalella azteca* for sediment. In the third stage, the biological evidence was evaluated as a response variable, composed of the richness data, Shannon-Weaver index, species evenness and numerical density of the zooplanktonic and benthic communities. In the final stage, the relationship between the explanatory and response variables was evaluated statistically. In this model, the ecological risk assessment was categorized as Low, Medium and High and was associated with the environmental quality of Excellent, Warning and Critical, respectively, established by the lines of evidence recorded at the sites monitored. The results showed that the investigative line of the chemical evidence, when compared to the lines of the ecotoxicological and biological evidence, clearly indicated signs of greater risk for both environmental compartments. The results enable the consideration that it is possible to use environmental monitoring programs for ecological risk assessment, if they contain, in addition to chemical and ecotoxicological data required by environmental legislation, monitoring data of the resident biota.

**MP188 Construction of Chemical Toxicity Prediction Model by Using Experiment Date, Mainly on Branched Nonylphenol Isomers** K. Arizona, Prefectural Univ of Kumamoto, Faculty of Env. & Symbiotic Science; H. Ji, Kumamoto Prefectural Univ; C. Sun, Kumamoto Prefectural Univ. The eighteen kinds of branched Nonylphenol (NP) isomers were used to build a Quantitative Structure-Activity Relationship (QSAR) model for predicting the toxicity of all the branched Nonylphenol Isomers. Acute toxicity evaluation of these 18 kinds of NP isomers was performed. The toxicity of these NP isomers was found relating to their chemical structure. Basing on the relationship between toxicity and chemical structure of these NP isomers, QSAR model was built for predicting toxicity of branched NP isomers. This QSAR model realized the toxicity prediction for all the branched NP isomer. In addition, toxicity effect evaluation of NP isomers mixture existing in the environment was performed by this QSAR model. The result showed that this QSAR model has a good applicability on evaluating the toxicity effect of NP isomers mixture existing in the environment.

**MP189 Crude Oil Impacts in an Amazonian Wetland** S.C. Peterson, Environmental Resources Management (ERM); K.A. Morris, D. Ross, H. Pirela, ERM; S. Stout, NewFields. An oil exploration and production facility located in an otherwise undisturbed area of the Amazonian lowland rainforest experienced releases of crude oil and produced water over a period of years, resulting in a large impacted area. Historical and recent aerial photographs and satellite imagery were used to define the extent and progression of the impact and subsequent natural recovery. This evidence, along with on-site observations, indicated that the oil was highly weathered, and the majority of the Site was undergoing significant natural re-vegetation of the impacted soils by both native and non-native wetland flora. To guide planning for an accelerated remediation and restoration of the Site, a comprehensive investigation and an ecological risk assessment was performed. Soil, surface water, and sediment samples were collected along transects in several distinct areas of the Site, as well as from offsite non-impacted areas, and submitted for detailed analysis of petroleum hydrocarbons using forensic chemical techniques. The chemistry results were used to identify and evaluate the degree of weathering of the crude oil, and to assess the potential bioavailability and ecological risks from residual constituents such as TPH fractions and PAHs. Samples were also submitted for microbial analysis to evaluate the presence and level of activity of petroleum degrading bacteria. In conjunction with the laboratory analyses, a field investigation was conducted to characterize vegetative diversity and vigor of plant growth. The chemical and microbial data confirmed that the oil is weathering and degrading due to the activity of a consortium of indigenous microbes, and significant mass removal of petroleum hydrocarbons is occurring naturally. The more bioavailable, lower carbon range fractions of petroleum hydrocarbons have been largely eliminated, leaving only a weathered and tarlike residual that is unlikely to cause significant toxicity to native fauna and flora. Parameters of vegetative succession were correlated with the degree of petroleum degradation, showing the potential for plant and animal communities to return to a more natural condition. Based on these results, the concept of exposure zones was used to identify areas of varying risk and to provide a framework for remedial planning.

**MP190 Current Conditions of *Pteria sterna* (Gould, 1851) in Bahía de la Paz, Baja California, Mexico** P. Ramirez Romero, Universidad

Autonoma Metropolitana, Hidrobiología, U.A.M. Iztapalapa, Depto. De Hidrobiología; M.G. Miranda Arce, L. Espejel Pina, X. Guzman Garcia, I. Hernandez-Calderas, G. Barrera Escorcia, Universidad Autonoma Metropolitana, Hidrobiología; I. Wong Chang, C.L. Fernandez Rendon, Universidad Nacional Autonoma de Mexico, ICMYL. *Pteria sterna* (Gould, 1851) is a protected species that produces high quality pearls. Even though there are some investigation about its culture, the decline of numerous populations due to environmental deterioration implies the need of integral studies to detect the effects of human activities on this resource. The objective of the present work was to obtain an histological description of the clam and to describe the concentrations of some contaminants that may affect them (metals and fecal coliform bacteria). Clams, water and sediments were collected in Bahía de La Paz in 2010. Histological analysis followed standardized methods, routine Hematoxyline-Eosine stain and PAS and Masson histochemical techniques. Cd, Cu, Cr, Pb y Zn concentrations in sediments were determined with an Solar AA spectrophotometer. Sanitary quality was evaluated following Mexican standards and enteric bacteria identification was done through traditional culture. Some histological characteristics indicated good conditions of the clam, like the male gonads; however the presence of mucopolisacárids and fibers suggest defense reactions in response to environmental stressors. Metal sediment concentrations do not represent any risk for the clam, only cadmium was above sediment guidelines during the rainy season in the site called La Sirena. Microbiological analysis indicated that the water sanitary quality occasionally exceeded the quality guidelines, but the clam had an acceptable quality for consumption. *Escherichia coli* was isolated from clams, *Salmonella* spp. from sediments and *S. choleraesuis* from water, this implies the need for systematic monitoring.

**MP191 Development of Evaluation Approach Towards Ecological Effect of Gaseous Sample on *Orius strigicollis*** S. Komatsu, R. Shichi, Tokyo National College of Technology, Chemical Science and Engineering; R. Shoji, Tokyo National College of Technology. Dichlorovos (dimethyl dichlorovinyl phosphate) is one of the most widely used volatile organic pesticide known for its high toxicity and volatile behavior. Though, there are many toxicity tests for dichlorovos were done in aqueous solution, in gaseous environment is yet to be developed. In this study, *Orius strigicollis* were used as a test organism to determine the toxicity of dichlorovos under vaporized condition. Also, authorized methods such as ROTAS® (Rapid Onsite Toxicity Audit System) and acute toxicity test using *Artemia salina* were conducted for comparison. As for result, toxicity test using *Orius strigicollis* showed similar sensitivity to those authorized methods. Therefore, toxicity test using *Orius strigicollis* can be a promising toxicity test on volatile organic pesticide.

**MP192 Development of Site-specific TCDD Bioaccumulation Equivalency Factors for Estimating Chlorinated Dioxin TEQs in Discharges to San Francisco Bay** S. Klosterhaus, D. Yee, J. Ross, San Francisco Estuary Institute. The use of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) bioaccumulation equivalency factors (BEFs) has been proposed for consideration in the determination of chlorinated dioxin toxic equivalent (TEQ) concentrations in wastewater effluent. BEFs, which have been adopted for use by the USEPA to account for differing levels of bioaccumulation potential among dioxin and furan congeners, have been derived using fish tissue and sediment data from the Great Lakes and are assumed to be representative of all ecosystems. In this study, TCDD BEFs derived from dioxin concentrations in two species of San Francisco Bay sport fish (white croaker and shiner surfperch) and surface sediments collected in 2009 were compared to the BEFs derived from the Great Lakes data. Both sets of BEFs were applied to San Francisco Bay wastewater effluent data to assess differences in the resulting TEQ concentrations. The dioxin biota-sediment bioaccumulation factors (BSAFs) used to derive BEFs for San Francisco Bay will be presented and discussed along with the BEF and TEQ results. Results from this work will contribute to the development of a water quality management strategy for dioxins (e.g., TMDL), which is currently being considered for San Francisco Bay.

**MP193 Differential Body Burdens of Various Compounds in Co-Occurring Marine Bivalves** J. Cura, Woods Hole Group, Risk Assessor, The Science Collaborative, Risk Assessor; J. Occhialini, Alpha Analytical; D. Vorhees, The Science Collaborative North Shore. Research over the past ten years has demonstrated that bioaccumulation of chemicals depends variously on the chemical properties, the species of organism, temporal differences,

environmental conditions, and the exposure history of an organism. There is ample evidence that under field and laboratory conditions, contaminant concentrations in tissue are often species-specific. For example, investigators have observed species differences in bioaccumulation of: Zinc between two filter feeding co-located epibenthic organisms (mussels and barnacles); PCBs between a deposit feeding and a filter feeding bivalve in laboratory uptake experiments; Various metals and PCBs between mussels and oysters observed in long term regional monitoring data in San Francisco Bay; Cadmium and copper among grass shrimp, mussels, and quahogs in controlled multi-element laboratory exposures; PAHs among various benthic species. These authors comment upon the implications of differential uptake and accumulation when selecting organisms for toxicity testing, bioaccumulation testing, or monitoring. These differences pose an uncertainty of generally unknown magnitude in ecological and human health risk assessments which often depend upon a small number of representative species. Risk assessors commonly select representative species to represent various trophic levels or vulnerabilities (ecological risk assessors) or ingested food types (human health risk assessors) with uncertain knowledge regarding the range of differences in bioaccumulation that may occur even among species of the same feeding type or taxonomic family. This work measures the range of tissue concentrations for various metals and organic chemicals among co-located bivalves that are both prey for local animal species and a regular food source for recreational shell fishers. We discuss the implications for selecting representative species in ecological risk assessment and selection of recreationally caught species in human health risk assessment.

**MP194 Differential mRNA Expression of Metallothionein and Vitellogenin in *Chironomus riparius* Larvae Exposed to Various Environmental Pollutants** K. Park, Chonnam National Univ, Fisheries and Ocean Science; I. Kwak, Chonnam National Univ. We characterized the metallothionein (MT) and vitellogenin (VTG) cDNA in *Chironomus riparius* and evaluated their mRNA expression profiles following treatment with different environmental pollutants. The objective of this study was to identify the possibility for the use of *Chironomus* MT and VTG as biomarkers of stress caused by endocrine-disrupting chemicals (EDCs), heavy metals, herbicides and veterinary antibiotics. The gene expression analysis showed that the MT mRNA levels increased significantly after long-term exposure to cadmium (Cd), copper (Cu), Lead (Pb), di(2-ethylhexyl) phthalate (DEHP), and 2,4-dichlorophenoxyacetic acid (2,4-D). Additionally, down regulation of the MT mRNA was observed in *C. riparius* exposed to 4-nonylphenol (NP) and fenbendazole (FBZ). However, bisphenol A (BPA) treatment did not activate the MT mRNA level. Moreover, the VTG mRNA expression increased significantly in *C. riparius* larvae exposed to BPA, NP, DEHP, Cd, 2,4-D and FBZ. Evaluation of the long-term effects of environmental pollutants revealed up regulation of *Chironomus* MT mRNA in response to DEHP exposure of the EDCs, and the level of the VTG mRNA was increased significantly following treatment with Cd and herbicide 2,4-D at all concentrations in a dose-dependent manner. These results indicate that MT and VTG could be used as potential biomarkers of herbicide and veterinary antibiotics as well as heavy metals and EDCs in aquatic environments.

**MP195 Does Size Matter? Accounting for Scale of Use in Bird and Mammal Risk Assessment** M. Reed, Chemicals Regulation Directorate; J. Crocker, Food and Environment Research Agency. Current approaches to regulatory risk assessment for birds and mammals in Europe are based on exposure of animals visiting treated crops. The assessment is the same for minor and major crops. Whilst at the single field scale the risk is the same at the regional scale the area a pesticide could be used would have an effect on the level of risk to the regional population. Where a risk assessment for a major crop shows an acceptable risk but for a minor crop it does not it is necessary to decide whether the small area treated can count in mitigation for the product's use on a minor crop. The aim of this poster is to present 3 approaches to including the area of crops in the risk assessment: 1. Extrapolation from the major crop to the minor crop. If the minor crop is similar to the major it may be possible to adopt the refined risk assessment from the major crop for the minor crop. 2. A rule of thumb setting the area below which pesticide effects on birds or mammals would be acceptable. This relies on being able to determine this area. It could be a rule in which a scale of use that would affect e.g., < 1% of arable land is authorised. 3. Isoclines describe an acceptable level of pesticide effects on populations and show how the corresponding acceptable area of pesticide use varies with the TER calculated in the risk assessment. This approach

combines both severity of effects and the area affected. Isoclines predict realistic worst-case effects of a pesticide on mortality and breeding success and place these local effects into a broader context, predicting effects at the population level. The isoclines have been drawn using the output from population models. Current decision making uses the first approach where possible. If extrapolation is not possible an alternative is required. The rule of thumb accepts as a premise that local effects on bird or mammal populations could occur (up to failure to breed successfully) but that these would be over a small enough area not to effect the population on a regional scale. This is not in line with current risk assessment which aims to protect the edge of field population. The isoclines approach also accepts that there could be some effects on local populations. The use of population models attempts to quantify the level of effect to allow decisions to be based on the level of risk and the area affected. There remains a question over whether any level of effects can be accepted for a species of bird that is already in decline.

**MP196 Evaluating the Ecotoxicity of American Petroleum Institute-sludge, a Petrochemical Waste, in Various Soil Types Using Three Soil Organisms** M. Van Wyk, Univ of Stellenbosch, Department of Botany and Zoology, Univ of Stellenbosch, Dept of Botany and Zoology; S.A. Reinecke, Univ of Stellenbosch; A.J. Reinecke, Univ of Stellenbosch, Dept of Botany and Zoology – Stress Ecology Research Group; R. Albertus, Sasol Technology. American Petroleum Institute (API) -sludge consists of a mixture of oil and water soluble contaminants originating from the process of separating refinery waste from reusable water and oil. To establish the soil toxicity of such API-sludge to soil organisms, different standardized soil types as well natural soil obtained from a South African refinery was used. Bioassays together with chemical analyses of the API-sludge were executed to thoroughly investigate the effects of different contaminants present in the mixture and its bioavailable fraction. Two standardized soil types, OECD soil and LUFA2.2 soil, were used in these experiments. A third natural soil, obtained from a site close to a South African refinery, was also included in this study to represent a soil type with potential risk of being contaminated by such petrochemical wastes. This soil was analyzed physically and chemically and included in the bioassays. Three soil species were used in the standardized tests: Two Oligochaetes, *Eisenia andrei*, *Enchytraeus doerjesi* and a Collembolan species, *Folsomia candida* were exposed to the respective soils to study their survival, growth (for *E. andrei*) and reproduction success. *F. candida* showed juvenile production of  $479.89 \pm 30.42$  where as *E. doerjesi* produced only  $57 \pm 34.39$  juveniles in the natural soil. *E. andrei* produced  $18.5 \pm 9.7$  cocoons when exposed to the natural soil. To determine the sensitivity of the organisms to the API-sludge, they were exposed to a concentration series of API-sludge-spiked control soils. The effect concentrations were calculated as the concentration of API-sludge that will decrease the studied endpoints by 50% of the control soils ( $EC_{50}$ ). The  $EC_{50}$ s varied for each species exposed in the different control soils showing that the toxicity of the API-sludge is to a certain extent dependent on the physical soil properties. The reproduction of *F. candida* were most sensitive to the API-sludge in natural soil ( $EC_{50} = 90$  mg/kg) and the *E. doerjesi* the least sensitive in LUFA2.2 soil ( $EC_{50} = 36000$  mg/kg). From the chemical analysis can be suggested that it is the volatile organic carbon fraction in the API-sludge that is responsible for its toxicity.

**MP197 Evaluating the Human Relevance for a Potential Carcinogenic Mode of Action for Naphthalene – an Hypothesis-based Weight of Evidence (HBWoE) Approach** L. Bailey, J. Goodman, L. Rhomberg, Gradient. Human health risk assessment consists of bringing to bear a large body of in vitro, animal, and epidemiologic studies on the question of whether environmental exposures to a substance are a potential risk to humans. However, the body of scientific information is typically unclear and often contains apparent contradictions; therefore, often only possible conclusions about potential human risks may be surmised from the data and may vary from very strong to tenuous. The task, therefore, is to also effectively communicate the uncertainties within the data, giving proper consideration to contrary data and alternative scientifically plausible interpretations. The National Toxicology Program (NTP) inhalation bioassay data for naphthalene showed an increased risk for nasal tumors in rats and lung tumors in mice, and although cytotoxicity and hyperplasia were observed in mouse nasal and to some extent in rat lung tissue, tumors were not observed in these tissues. CYP2F metabolism of naphthalene to its epoxide and cytotoxicity has been invoked as a potential carcinogenic mode of action (MOA) for naphthalene, and there is CYP2F activity in

mouse lung and in rat and mouse nasal epithelium. The lack of mouse nasal tumors, despite the observation in the mouse nose of other key events in the proposed MoA, needs to be accounted for, and the weight of evidence for the proposed MoA (and its application to potential human risk) depends on the degree to which a supportable explanation can be identified that is in accord with known science and makes sparing use of post-hoc assumptions to accommodate the apparent discrepancy. We evaluate the available data for naphthalene, through the Hypothesis-Based Weight of Evidence (HBWoE) approach, considering potential key events in the MOA in each species and tissue: metabolism by CYP2F, cytotoxicity, chronic inflammation, potential genotoxicity, regenerative hyperplasia, and tumor formation. We evaluate the weight of evidence in support of a cytotoxic MOA, in addition to alternative MOAs that have also been offered in the scientific community, noting the explanatory power of the proposed hypotheses, considering consistencies, inconsistencies, and contradictions within the data set, and how compelling each endpoint is as evidence of a similar MOA in humans from low levels of naphthalene exposure.

#### MP198 Generic Population Models for Ecological Risk Assessment

N. Hanson, Univ of Gothenburg, Dept of Plant and Environmental Sciences, Univ of Gothenburg, Plant and Environmental Sciences; J.D. Stark, Washington State Univ, Puyallup Research and Extension Center, Dept of Entomology, Washington State Univ, Dept of Entomology. It has been argued that current approaches for ecological risk assessment (ERA) do not provide value relevance for risk managers, and that the uncertainty in the predictions is large. One important reason for this is that current approaches are based on individual level endpoints, while the environmental protection goals are found on the population level. Population models may be a useful tool to link the individual with the population, and thereby increase value relevance and reduce uncertainty. However, population models are often considered too complex compared to traditional measures of acute mortality and reproductive effects. Therefore, simple models, and guidance on how to use them, needs to be made available for risk assessors. Only if such models are available can population models gain momentum in ERA. In the present paper, generic models were developed for ERA of fish populations. Fish have high ecological and economic value, and are frequently used in ERA. It is, therefore, highly desirable to develop better methods for ERA of fish populations. The models were based on five types of life-histories, and they were set up to be useful in a number of different management scenarios based on different environmental protection goals. A decision framework was set up for three Tiers of models, and three levels of environmental protection. Data from previously published studies were tested at Tier I for two of the environmental protection levels. The models provided maximum acceptable concentrations (MAC) that fell between the MACs of traditional ERA based on acute or chronic data, respectively. Although this does not prove that the models provide better risk assessments, it clearly shows that traditional methods are likely to lead to erroneous estimates of safe limits (either over- or underprotective).

**MP199 Hazardous Pollution in Tien-Shen** L. Hadjamberdiev, Toxic Action Network Central Asia. There was Uranium Boiler of former USSR in Tien-Shen-Pamir areas, especially near settlements Adrasman, Ak-Tjuz, Chkalovsk, Kadji-Say, Mailuu-Suu, Min-Kush, Taboshar. There were obsolete methods of uranium wastages had been used in 50th. Uranium waste pulp had been kept in the mountain gorges, coved by concrete with sands and soil, lie about 6-10 m only. There are tenth old tailings and dumps with other toxicants (mercury, cyanide, etc). Tremendous Mailuu-Suu uranium mining area (West Tien-Shen), 23 tailings with total volume 2 mln m<sup>3</sup>, contaminated river Mailuu-Suu water – uran 250-750 mg/L. Khaidarken (the mercury combine production volume was second in the World) situated in South-West Tien-Shen. The wastes contain (volume 4 mln tones), beside Hg, fluoric ore elements (Ar, Sb, F compounds). The tailings and dumps permanent contaminated living area. Additionally, tailings are situated on height of 1700 m above sea level near river Zarhar, and can produce pollution of neighboring territory of Uzbekistan. Cyanide problem: gold combine in Tien-Shen produced biggest in Asia toxic tailing, 100 mln cubic m, but new lake situated upper the dump (which saved the tailing), can destroy dump, and wastes will flow down to river Arabel and Narin. Several big uranium tailings polluting during latest ten years, and could produce danger pollution of territory by throw down to rivers: 1) MinKush uran mining area (Central Tien-Shen), Tujuk-Suu tailing can be pour out to the rivers net Min-Kush – Kokomeren – Naryn and Toktogul water deposit; infiltrating

throw dam steadfast increasing last 5 years; 2) North Pamir – Degmay uranium tailing – in cost of river Karamazar-Say, and 8 km near main CA Syr-Darja river; 3) Radioactivity in several points (former building of Adrasman) 160 mkR/hour. We study uranium and mercury pollution of the rivers water, groundwater, vegetables in the rivers cost. The health consequences (heritage and cancer diseases) due uranium and mercury exposure in the areas have been documented several years.

#### MP200 In Vitro Screening of 1,2- Dibromo-4-(1, 2-dibromoethyl) cyclohexane in Multiple Human Cancer Cell Lines for Possible Endocrine Disrupting Effects

H. Scherr, Simon Fraser Univ, Biological Sciences; T. Beischlag, Simon Fraser Univ, Health Sciences; J.E. Elliott, Environment Canada, Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada, Pacific Wildlife Research Centre, Environment Canada, Canadian Wildlife Service. Many brominated flame retardants (BFRs) can be classified as endocrine disrupting compounds. Various health problems in both humans and animals have been attributed to BFRs. One BFR, TBECH (1,2- dibromo-4-(1, 2-dibromoethyl) cyclohexane), has recently been detected in the environment and biota, but very little is known of its environmental impacts. To identify whether or not TBECH has any endocrine disrupting capabilities, it was run through an in vitro screening process that included real-time PCR techniques in two different cell lines (human endometrial carcinoma cell line ECC-1, and human prostate cancer cell line LNCaPs), and use of the CALUX assay in the human breast cancer cell line T-47Luc4ARE (Androgen Response Element CALUX). We were interested in whether or not TBECH was able to cause expression in human androgen, estrogen, progesterone, and aryl hydrocarbon receptors. All cells were treated with environmentally comparable levels of TBECH alone and in combination with the respective steroid hormones to determine if the compound acts as an agonist, antagonist, or amplifies the effects of the hormones. For the aryl hydrocarbon receptors, cells were treated with environmentally comparable levels of TBECH alone and in combination with TCDD, a xenobiotics known to activate this receptor. RNA extraction and real-time PCR techniques were used to measure any gene expression that occurred due to activation or inhibition of the various receptors in ECC-1 and LNCaP cell lines. For the T-47Luc4ARE cell line, androgen expression was measured by the concentration of proteins produced from activation of the androgen receptor. Initial results indicate that TBECH does not act as an agonist in any of the receptors tested, however dihydrotestosterone activation potential is significantly decreased when in combination with TBECH in the T-47Luc4ARE cell line. These tests were meant to be preliminary steps in order to determine endpoints that will be examined more closely later on in a live zebra finch model.

#### MP201 Influence of Species-specific Attributes on Dietary and Tissue-based Exposure Assessments of Two Fish-eating Bird Species Exposed to PCDFs and PCDDs

R.M. Seston, Dow Corning Corporation, Health & Environmental Sciences; T.B. Fredricks, Monsanto, Zoology; D. Tazelaar, Michigan State Univ, Animal Science, Cardno Entrix; W.R. Folland, M.W. Nadeau, P.W. Bradley, Michigan State Univ, Dept of Animal Science; J. Newsted, D. Kay, Cardno Entrix; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; M.J. Zwiernik, Michigan State Univ, Dept of Animal Science. Selection of receptor species is a critical step in formulating the study design of ecological risk assessments (ERAs). The great blue heron (*Ardea herodias*; GBH) and belted kingfisher (*Ceryle alcyon*; BKF) are piscivorous predators that are often selected as receptor species for ERAs pertaining to persistent bioaccumulative contaminants in aquatic environments. Both species have similar dietary compositions, however their exposure characteristics including metabolic rates, foraging range, and site fidelity may differ. Matching receptor characteristics to site attributes and risk management goals ultimately determines the value of any ERA. The primary objective of the present study was to investigate the influence of species' life history characteristics in the selection of the most effective receptor. Sediments and floodplain soils of the Tittabawassee River (TR) downstream of Midland, MI, USA contain total concentrations of the seventeen 2,3,7,8-substituted polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) ranging from 1.0x10<sup>2</sup> to 5.4x10<sup>4</sup> ng/kg dw. These concentrations of PCDD/DFs in sediments and soils are some of the greatest reported and are 10- to 20-fold greater than the regional background. As apical avian piscivorous predators, GBH and BKF were selected as aquatic-based receptor species within a broad group of receptors in support of an ERA. Both were



considered appropriate species for inclusion based on their presence on-site and great potential to experience elevated exposures to bioaccumulative PCDD/DFs. Exposure for both the GBH and BKF was quantified by predicting dietary exposure to contaminants and direct measurement of contaminants in tissues of each species. The degree to which these two lines of evidence converged for each species was evaluated by comparing biomagnification factors (BMFs) of select congeners between the two species. Ratios of site-specific predominant PCDD/DF congeners indicate that both species were foraging within the TR floodplain. However, BMFs for the GBH are lesser than those for the BKF. Differences in BMFs may stem from either species-specific metabolism or foraging behavior. Concentrations of PCDD/DFs in BKF tissues were greater than those observed in GBH, suggesting the restricted foraging range of BKF within 1 km of the nest burrow offers greater spatial resolution and maximal exposure compared to GBH.

**MP202 Integration of Ecological Assessment in Computing Service Loss Thresholds** M. Schuck, Geosyntec Consultants, Sr. Staff Risk Assessment Scientist, Geosyntec Consultants, Risk Assessment Scientist; A. Brandon, K. Kroeger, H. Cumberland, J. Tolson, Geosyntec Consultants. Estimating service loss for natural resource damage assessments (NRDA) under a habitat equivalence analysis (HEA) approach requires numerical service loss thresholds to be established for each chemical stressor impacting the ecosystem. For example, sediment PAH levels of 1 and 10 ppm could be established for service loss thresholds of 5 and 20 percent, respectively. These service loss thresholds are often developed through a literature review of toxicity studies linked to ecological effect levels (e.g., LOAEL, NOAEL). Cumulative effects from multiple stressors are then calculated by a multiplicative process in which the percent service loss from each individual stressor is applied successively to the remaining service level after accounting for other stressors. The process results in a service loss that can range from 1 to 100 percent and is reflective of cumulative effects based on literature-derived effects levels. However, calculated theoretical service losses based on effects levels are often not supported by site-specific evidence collected for CERCLA ecological assessments. In addition to concentration-based comparisons, ecological assessments utilize in situ toxicity studies and benthic and fish community assessments to evaluate the health of the ecosystem. A novel approach is proposed that calculates cumulative service loss in the context of the results of the ecological assessment. The results from the ecological assessment are utilized to establish a limit of potential service loss. Services loss estimates within the established limits are then calculated using the existing framework for concentration-based assessment and cumulative effects calculation. This novel approach provides a more realistic estimation of service loss and additional accuracy in the process for assessing natural resource damages.

**MP203 Mitochondrial Dynamics and Autophagy Aid in Removal of Persistent Mitochondrial DNA Damage** A.S. Bess, T. Crocker, I. Ryde, J.N. Meyer, Duke Univ, Nicholas School of the Environment. Mitochondrial DNA (mtDNA) integrity is critical for human health; however, it is unclear how persistent, helix-distorting mtDNA damage formed after exposure to environmentally important genotoxins such as ultraviolet radiation, mycotoxins and PAHs are handled. mtDNA may be particularly susceptible to these genotoxins due to the absence of nucleotide excision repair, the primary repair mechanism for such DNA damage in nuclear DNA. Mitochondrial dynamics and autophagy play key roles in maintenance of mitochondrial function and degradation. We investigated the removal of persistent mtDNA damage via mitochondrial fusion, fission and autophagy in *Caenorhabditis elegans*. We performed RNAi knockdown of mitochondrial fusion, fission and autophagy genes in UVC treated adult *glp-1* *C. elegans*. Using a quantitative PCR assay, we observed significant removal of UVC-induced mtDNA damage in empty vector controls following five day recovery from exposure. Knockdown of autophagy, fusion and fission genes inhibited removal of UVC-induced mtDNA damage. These data indicate that autophagy, fusion and fission processes are required for the removal of persistent DNA damage in mitochondria in *C. elegans*. To better understand the mechanism by which mitochondrial dynamics aid in removal of persistent mtDNA damage, we investigated changes in mitochondrial dynamics (fusion/fission) following UVC exposure in mouse embryonic fibroblasts (MEFs). MEFs were transfected with mitochondrial matrix-targeted photoactivatable GFP (PA-GFP) and diffusion of PA-GFP through the mitochondrial network was measured following 405 nm laser activation of PA-GFP within a subset of mitochondria. UVC exposure significantly

increased GFP diffusion in the mitochondrial network indicating an increase in mitochondrial fusion and/or a hyperfused mitochondrial morphology. Therefore, we propose a model whereby UVC-induced mtDNA damage is removed via fusion-mediated mitochondrial remodeling and subsequent fission and autophagy.

**MP204 Multiple Line of Evidence Assessment of Sediments from Southern California Using California's New Sediment Quality Objective Protocols** D.J. Greenstein, Southern California Coastal Water Research Project, Dept of Toxicology; S.M. Bay, Southern California Coastal Water Research Project, Toxicology Dept; K. Schiff, K. Ritter, A. Ranasinghe, Southern California Coastal Water Research Project; L. Tiefenthaler, Southern California Coastal Water Research Project, Watersheds. The California Sediment Quality Objectives (SQO) assessment framework was approved for use in 2009 to assess sediment quality in the state's enclosed bays and estuaries. The SQO framework uses sediment chemistry, toxicity and benthic community analysis in a multiple line of evidence approach. Each line of evidence has multiple endpoints and interpretation thresholds that were developed using novel statistical methods. The Southern California Bight 2008 Regional Monitoring Program (Bight'08) represents the first large-scale study designed to utilize the SQO framework. Assessments were performed on approximately 180 stations located in predominantly marine bays and estuaries. The random stratified sampling design of Bight'08 allowed for the calculation of the total area and percentage of area that fell into five assessment categories for four different strata (Bay, Marina, Port and Estuary). Estuary sites generally had the greatest magnitude and prevalence of chemical exposure and biological effects. Temporal trends in sediment quality over the last decade, as well as predictive relationships between the sediment toxicity or chemistry lines of evidence and benthic community impacts were also examined.

**MP205 Next Steps in Endangered Species Risk Assessments** C. Habig, Exponent, Inc., Compliance Services, Inc., Principal Scientist. As an integral part of its registration review program, EPA is conducting screening level endangered species risk assessments for pesticides. These assessments, which evaluate potential effects on endangered birds, non-target mammals, fish, aquatic invertebrates, and non-target plants, typically consist of EPA's standard, national-level screening risk assessments for pesticides. These screening-level assessments are inherently intended to be conservative, so if a pesticide-use combination passes this initial assessment, there is a relatively high degree of confidence that those uses of the product will not adversely impact endangered or threatened species. However, if the use of a product fails this initial assessment, the conclusion should not be that use of the product is likely to adversely impact endangered species; rather, the conclusion should be that the risk assessment should be refined. However, EPA does not conduct a refined assessment. Instead, those uses of the product that fail the initial assessment may be referred to the Fish and Wildlife Service (FWS) or the National Marine Fisheries Service (NMFS; collectively, the Services) for further evaluation in a Biological Opinion (BiOp). The Services approach to risk assessments of pesticides differs considerably from the approach that EPA and registrants typically use. Therefore, registrants are encouraged to conduct a higher-tier risk assessment to evaluate the potential for uses of a product that fails a screening level risk assessment to impact endangered species. A higher-tier risk assessment can involve several levels of refinement. This paper provides examples of several types of refinements, including more detailed, specific use information, more specific information relating to endangered species that may be impacted, refinements in potential exposure, and refinements in toxicity endpoints, that can be used to address endangered species concerns.

**MP206 Spatially Defining Relationships Between ESA-listed Species and Pesticide Uses in Agricultural Crops** A. Frank, Compliance Services International, Principal Consultant; G. Konkel, Compliance Services International; T. Hall, Bayer CropScience, Ecotoxicology. In the conduct of FIFRA risk assessments related to evaluating the potential risk to species listed by the Endangered Species Act (ESA), the Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP) relies upon various sources of data, including spatial and temporal data that can help define "proximity" of a known species location or habitat to potential product use. Additionally, the Services (US Fish & Wildlife Service and National Marine Fisheries Service) are interested in the spatial representation of a crop when FIFRA endangered species matters are forwarded to them for consultation.

When a proposed use is associated with a given crop, spatial definition of where that crop occurs is achievable through various mechanisms: the soil or climate conditions required to support the crop, USDA Agricultural Statistics on where the crop is, or has been, grown, and land cover data on the distribution of the crop, as provided through USGS, NASS and other resources. This poster will illustrate how different data sets can be used in the FIFRA risk assessment setting to spatially define relationships between ESA-listed species and proposed product use in agricultural crops. A process for defining these relationships in rotational crop systems, or in crop systems over time, will also be explored.

**MP207 Standardization of Wildlife Toxicity Reference Values Proves**

**Elusive** D.B. Mayfield, Gradient; A. Fairbrother, Exponent, EcoSciences, Exponent, Inc., EcoSciences. Wildlife toxicity reference values (TRVs) are routinely developed during screening level and baseline ecological risk assessments (ERAs). Risk assessment professionals often adopt TRVs from published sources in order to expedite risk analyses. The USEPA developed Ecological Soil Screening Levels (EcoSSLs) to provide a source of TRVs that would increase the consistency among screening analyses conducted for contaminated sites. We conducted a survey of publically available ERAs to evaluate uniformity in the use of wildlife TRVs across the United States. We evaluated more than 50 large-scale ERAs published in the last decade in order to understand current practices for wildlife TRV use and development within the risk assessment community. The use of no observed and lowest observed adverse effect levels (NOAELs and LOAELs) culled from published compendia was common practice among the majority of ERAs reviewed. We found increasing use of TRVs established in the EcoSSL documents; however, EcoSSL values were not used in the majority of the ERAs reviewed. There is still wide variability in TRVs selected for commonly studied contaminants (e.g., metals, pesticides, PAHs, and PCBs). Differences in selection of key studies used to derive the TRVs, in dose estimation, and in use of uncertainty factors result in TRVs that span multiple orders of magnitude for any one pollutant. This lack of consistency in TRV development leads to inconsistent and unbalanced remedial decisions on the national scale.

**MP209 The Influence of Reproductive Strategy of Fishes on Sampling Protocols for Environmental Monitoring Programs for Industrial Effluents in Canada**

T.J. Barrett, University of New Brunswick, Dept of Biology; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology; M.R. van den Heuvel, University of Prince Edward Island, Dept of Biology. National environmental effects monitoring (EEM) programs in Canada have been developed for the pulp and paper and metal mining industries and a program is currently in development for municipal wastewater. The EEM programs include a fish population survey to assess impacts of these effluents of fish populations. A review of the pulp and paper EEM program has identified many surveys were conducted outside of the optimal period to identify reproductive impacts. A review of the more than 60 different species used in the EEM fish surveys identified different female reproductive patterns based on the number of spawning events per year and the timing and duration of gonadal recrudescence in females. Examination of temporal stability, minimum variability, and maximum development in female gonad size over a reproductive cycle for species of each reproductive pattern revealed optimal sampling periods to assess reproductive impacts for each reproductive pattern. The variability in ovary weight: body weight relationships and the variability in oocyte development in pre-spawning female fishes in Atlantic Canada were also examined to identify patterns that could be used to predict the reproductive strategies of fishes. The optimal sampling times will reduce the variability in the reproductive endpoints for the EEM fish survey and hence reduce sample sizes for lethal sampling. Predicting the reproductive strategy of fishes will enable researchers to gather baseline information on the reproductive biology of fishes and therefore identify optimal sampling periods to identify reproductive impacts for species whose life history is not well studied.

**MP210 The Response of Benthic Organisms at Population and Community Levels Against Heavy Metal and Organic Pollution Gradients in Contaminated Sediments**

J. Khim, Korea Univ, Division of Environmental Science and Ecological Engineering; J. Ryu, Korea Ocean Research and Development Institute, Office of Policy Research; S. Kang, Korea Ocean Research and Development Institute, Maritime & Ocean Engineering Research Institute; C. Koh, Seoul National Univ, School of Earth and

Environmental Sciences. The effect of sediment pollution on benthic organisms was investigated in the vicinity of a large sewage treatment outflow at Incheon North Harbor, Korea. Animal size, vertical distribution and standard community parameters were analyzed along a 3 km transect line (n=7). Univariate parameters showed a general trend of increasing species diversity with increasing distance from the pollution source. Multi-dimensional scaling analysis led to the clear separation of 3 locational groups, supporting gradient-dependent faunal composition. The innermost location was dominated by small sub-surface dwellers while the outer locations by large mid to deep burrowers. Looking for the size-frequency distribution, most abundance species (*Heteromastus filiformis*) showed the presence of larger size animals with increasing proximity to the pollution source. Meanwhile, species-specific vertical distributions, regardless of the pollution gradient, indicated that such shifts were due to species replacement resulting from a higher tolerance to sedimentary pollutants over some species. Overall, the present study highlighted that community and population level response to the polluted environment of the harbour reflected an integration effect, together with biological interactions.

**MP211 The Responses of ERR Gamma Gene on *Chironomus riparius* Exposed to Endocrine-disrupting Chemicals**

K. Park, Chonnam National Univ, Fisheries and Ocean Science; I. Kwak, Chonnam National Univ. There is global concern with regard to the potential impacts of endocrine-disrupting chemicals (EDCs) on the aquatic environment. EDCs can interfere with the endocrine systems of a variety of organisms. In order to evaluate the effects of EDCs on the estrogen-related receptors (ERR) of *Chironomus*, we characterized full-length cDNA sequences of the ERR gene from *Chironomus riparius*. The complete cDNA sequence of the ERR gene was measured to be 1332 bp in length. The results of our phylogenetic analysis demonstrated that *C. riparius* ERR is most closely related to that of mosquitoes. The basal level of ERR mRNA was expressed abundantly during different life-history stages, with the exception of adult male. Additionally, ERR gene expression was induced to a significant degree in *C. riparius* exposed to bisphenol (BPA) and nonylphenol (NP) at all concentrations for short periods. After short periods of exposure to di(2-ethylhexyl)phthalate (DEHP), the response of the ERR gene was increased significantly at only 50 mg L<sup>-1</sup> DEHP. However, under long-term exposure conditions, ERR expression was induced to a significant degree after BPA, NP, and DEHP exposures at all concentrations. Furthermore, increased ERR expression was also noted in the results of Western blotting with ERR $\gamma$  antibody and on the luciferase reporter gene assay. Collectively, these findings indicate that EDCs influence the functions of ERR in *Chironomus* species.

**MP212 Upper Columbia River Sediment Toxicity Evaluation Using a Reference Envelope Approach**

C. Irvine, CH2M Hill, Ecosystem Services; E. Byron, CH2M Hill, Environmental Services; F. Dillon, CH2M Hill. Remedial investigations are ongoing to assess the potential risks to ecological receptors in the Upper Columbia River (UCR), WA, where contaminants, primarily metals, may be present in sediment at concentrations that pose an unacceptable risk to benthic/epibenthic resources. A sediment sampling and laboratory toxicity testing program was conducted to assess the toxicity to benthic/epibenthic resources from sediments. Over 387 samples were collected over the 150 mile Site for chemical analyses, 50 of which were also tested for toxicity. Toxicity testing included 10-day whole-sediment toxicity tests with the midge, *Chironomus dilutus*; 28-day whole-sediment toxicity tests with the amphipod, *Hyalella azteca*; and 7-day toxicity tests with the cladoceran, *Ceriodaphnia dubia*. A reference envelope approach, use of toxicity metrics (e.g., AVS-SEM, PEC-Qs, PW-TUs), and statistical analyses were used to identify relationships between sediment chemistry and sediment toxicity. At least one of the toxicity endpoints (i.e., survival, growth, biomass, or reproduction) for at least one test organism, was reduced relative to a reference envelope in 43 of 50 site sediment samples. However, only 16 samples differed from the reference envelope by what might be considered an environmentally relevant amount (< 80 percent of the reference envelope criteria). Statistically significant effects were observed in a total of 28 samples and occurred throughout the sampled length of the UCR. Effects were generally more prevalent closest to the US-Canada border and were more variable downstream. *Hyalella* growth and biomass were the most sensitive endpoints with the most number of samples with significant effects and with significant effects for all samples where effects were also observed for either Chironomids or *Ceriodaphnia*. Statistically significant but weak relationships existed between toxicity endpoints and metrics of sediment metals (i.e.,

SEM-AVS, mean PEC-Qmetals, summed PEC-Qmetals) and PEC-Qs for chromium, copper, and zinc. Zinc and copper PEC-Qs were the highest in most samples, suggesting that these two metals were drivers of adverse effects in sediment samples. The weight-of-evidence indicated that benthic/epibenthic resources are at risk. However, there was moderate uncertainty about these results because of the poor ability of the exposure metric-response / concentration-response relationships to predict toxicity.

**MP213 Use of the Plant Bioindicator *L. multiflorum* for Ecological Risk Analysis of Air Emissions** R.d. Magali, Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus, Environmental; K. Tallini, Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus, Biotechnology; M.T. Raya-Rodrigues, Federal Univ of Rio Grande do Sul, Ecology Center. Ecological Risk Analysis (ERA) is a tool used to assess the sustainability of ecosystems, allowing for a more accurate assessment of actual risk to ecological receptors affected by contaminants (Junger, *et al.*, 2005; Sousa, 2005; Nakagome, *et al.*, 2006; Arias, *et al.*, 2007; Niemeyer, 2007; Froehmer & Martins, 2008; Parkhurst, 1996). It is a scientific methodology for quantifying or perceiving the adverse effect risks of a pollutant on the environment (Mines & Lackey, 2009). The study was conducted in the vicinity of an oil refinery (January 2005 to March 2008) in Esteio (29°49'16" S 51°08'09" O), Rio Grande do Sul, Brazil. The aim was to develop and implement an ERA model in order to classify the risks associated with air emissions on the basis of using the bioindicator *Lolium multiflorum*. The latter associated the information from the effect of the stressors, derived from the atmospheric emissions of sulfur compounds, in the receptors, that is, in the tissue of the bioindicator. The percentage of accumulated sulfur was evaluated in the bioindicators, classified, in this study, as a response variable. This was integrated with the data of the SO<sub>2</sub> concentration in the atmosphere, which gave rise to the explanatory variables, toxic potential unit (TPU) and toxic exposure unit (TEU). TPU was defined as the quotient between the average concentration of SO<sub>2</sub> that exceeded the primary air quality standard and secondary air quality standard (CONAMA 03/90), indicating environmental concentrations of SO<sub>2</sub> that can affect the growth of the bioindicators through chronic effect. TEU was defined as the product of TPU and the exposure period of the bioindicators to a higher concentration than the primary air quality standard, indicating SO<sub>2</sub> concentrations that can affect bioindicators through acute effect. The classification TPU ≤ 1 unlikely ecological risk and TPU > 1 potential adverse effects were considered as evidence of risk. On the basis of the environmental quality data (Crittenden & Read, 1979; CONAMA 03/90; USEPA, 1989; Tarazona, 2000, Klumpp, 2001) the classes of ecological risk were established (low, medium and high). The integration between the explanatory and response variables on the basis of multivariate statistical correlation analysis with the use of SPSS software, version 13.0 for Windows, showed evidence of risk to vegetation surrounding the refinery when exposed to TPU greater than the unit.

**MP214 Using Web-based Tools for Data and Project Management** R. O'Boyle, C. Kessel, J. Ma, Exponent. The complexity of environmental projects and personnel involved have led to situations where data can be distributed across many organizations. This distribution of data and how it is handled at each level plays a significant role in the overall project evaluation and assessment. Distributed data can cause project delays, missed project deadlines, and excessive expenditures due to data contamination, the need to complete repetitive quality reviews, and loss of data, all of which could have regulatory impacts to the project. A database tool that is accessible to all project participants via web interface is one of the best avenues to get data and project information into the hands of potential users and decision-makers. This type of tool can be accessed from any location with internet access, allows for a central depository of all relevant project material, and can provide various levels of data access and retrieval. This poster will display a variety of web-based tools and concepts that can efficiently and effectively manage all aspects of a project (documents, data, schedules, personnel, etc).

**MP215 A Streamlined Risk Assessment Approach for Remediation of Industrial Facilities** T. Biksey, WSP Environment & Energy, Director Risk Assessment, EHS Support Corporation, Director Risk Assessment. The objective of the risk assessment process is to provide the risk manager with the necessary information to develop and evaluate remedial alternatives for corrective action at a site that are protective of human health and the environment with a reasonable foreseeable land use (e.g., continued commercial

or industrial activity). The basis for an acceptable potential risk can include incorporating engineering and institutional controls as part of the development of remedial activities. For example, institutional controls can be implemented for the restricted use of groundwater as a potable resource, or soil management operational plans can be used for current and future exposures that may occur due to soil disturbance activities in the paved areas (e.g., utility maintenance) or from excavation activities (e.g., new building construction). A streamline risk assessment framework was developed for application to an industrial facility that has the components of a baseline risk assessment, including current and future land use as an operating facility with commercial off-site uses, and a future hypothetical unrestricted residential use of the site. However, because the focus is continued industrial activity, the streamline risk assessment framework focuses on those potentially complete exposure pathways that will exist within the current and reasonable foreseeable land use. It is recognized that additional investigations and risk assessment may be necessary for future redevelopment of the site to meet unrestricted future use. A key focus of the framework is the selection of constituents of potential concern (COPCs). The COPCs are evaluated as potential risk-drivers based on the current and reasonable foreseeable land use. If the COPC is detected at a concentration greater than the risk-based screening criteria, but is not part of a complete exposure pathway associated with a reasonable foreseeable land use (groundwater used that is restricted as a potable resource), then the COPC is not evaluated further in the risk assessment process. The purpose of this streamline framework is to limit unnecessary sampling, analysis, and evaluation based on reasonable foreseeable land use, and to ensure that realistic remediation goals for human health and environmental risk-reduction are established.

**MP216 Best Practices Document for Application of Geostatistics to Sediment Assessment and Remediation** J. Schofield, CSC, CSC, Senior Environmental Scientist; P. Goovaerts, CSC, BioMedware, Inc; J.W. Kern, Kern Statistical Services, Inc. To support successful remedial projects, a rigorous approach is needed to accurately characterize sediment contamination, estimate sediment volumes, and evaluate achievement of clean up goals. For a number of historical projects such as the Hudson river, prediction of contaminated sediment volumes has been low biased which can result in projects being over budget, past schedule and ineffective, with no choice but to leave contamination behind. Geostatistical tools, originally developed for the mining industry, are being increasingly applied in sediment site characterization and proving useful to support many aspects of remedial projects. One benefit of geostatistics is that it provides a measure of the uncertainty of model estimates whereas with other methods decision makers are blind to the confidence bounds around the estimates, which might be very large. Although the geostatistical methods have been used in mining for decades and are being applied to a variety of new fields, several challenges arise during application. Geostatistics have not been widely applied to sediment projects, few publications on the effectiveness for these types of projects are available, and specific expertise is not common. Standard practices have not been developed and, as is the case with many statistical tools, proper use of geostatistical tools is critical to the accuracy of results. To address these challenges, a best practices document for application of geostatistics to sediment projects is being developed by the US Environmental Protection Agency. The document will detail critical processes (e.g., data distribution evaluation) for proper application of geostatistical tools. Because different stages of a project require different levels of rigor, the document will provide guidelines for selection of the most appropriate tool for each step. The guidance also will detail reporting and documentation requirements to facilitate reproducibility of model results. The availability of a best practices document will facilitate use of the best available tools to support remedial decision making and result in more accurate contamination characterization and volume estimates. The tools would be applied according to best practices while contributing to the creation of a pool of expertise that is currently lacking in the field.

**MP217 CHiPs – Industrial Process Hazard Assessments for Potential Chemical Releases** L. Barber, C. Hard, C. Rudisill, SRC, Inc.; T. Miller, J. Rhodes, R. Garret, Dept of Defense; J. Tunkel, SRC, Inc. The Dept of Defense performs contingency assessment activities on chemical hazards utilizing a suite of database-driven tools. The Chemical Hazard of Industrial Processes (CHiPs) database contains information on industrial processes that use or produce commercial chemical substances. In its original form, the CHiPs reference database was designed to aid in the analysis of the



commercial chemical substance production and infrastructure. This presentation provides an overview of the methodology that was developed for a new CHIPs product, Industrial Process Hazard Sheets (CHIPs-IPHS), that were designed to communicate the hazards associated with the large scale release of chemicals from facilities where they are manufactured, used, or stored. The CHIPs-IPHS methodology was developed to assess both the potential health (toxicological) and physical hazards for all chemical substances that are present at a facility. The challenge in developing the CHIP-IPHS methodology was to define the perspective required to adequately categorize hazards for liquids, solids, gases, and solutions associated with the full variety of industrial processes, including chemical-based manufacturing and mechanical-based processes that use chemical substances, as well as non-manufacturing facilities where they are present. CHIPs analyses are based primarily on data collected from public domain resources including quality compilations such as SRC's Environmental Fate Database (EFDB) and commercial subscription services. To account for the likelihood that a health or physical hazard may be encountered, a probability score is defined by assessing volume on site, volatility, and the potential for chemical release within the scope of each process. Preliminary health and physical scores are then obtained using bright-line criteria to address a chemical's toxicity and flammability or instability, respectively. Pairing these two sets of scores in a matrix reveals an initial hazard for toxicity and physical hazards (high, moderate, low, or negligible) that incorporates the uncertainties noted during the assessment. The resulting hazard for each chemical is the higher of these two values, and the overall process hazard is obtained from the chemical with the highest level of concern. Examples of the decision logic used in CHIPs-IPHS, as well as the results of industrial processes scored under this methodology, will be provided.

**MP218 Ecological Disinfestation: Evaluation of Substrates to Filter Zoospores of *Phytophthora nicotianae* from Water** E. Nyberg, Clemson Univ, Institute of Environmental Toxicology; I. Meadows, S.N. Jeffers, Clemson Univ, Entomology, Soils, and Plant Sciences; S.A. White, Clemson Univ, Horticulture, Clemson Univ, Clemson Institute of Environmental Horticulture. Developing an ecologically-based remediation system to remove propagules of plant pathogens, particularly species of *Phytophthora*, from water at ornamental plant nurseries has the potential to reduce the need for agrichemical applications and, therefore, should decrease pesticide residues entering the environment. Focusing on irrigation water as a point-source of pathogen inoculum, the goal of this project was to evaluate and characterize the effectiveness of five substrates (sand, crushed brick, calcined clay, Kaldnes medium, and polyethylene beads) to filter zoospores of *Phytophthora nicotianae* from water. The physical filtration capability of each substrate was examined by pumping a zoospore suspension (10 zoospores/ml) through PVC columns (5 cm in diameter); six replicate columns were filled with each substrate, and each substrate was tested at six depths: 0, 5, 10, 20, 40, and 60 cm. Influent and effluent samples of suspension were collected from each column. Zoospore densities were quantified by filtering 200-ml aliquots through polycarbonate membranes (47 mm in diameter, 3- $\mu$ m pores) and allowing colonies to grow on PARPH-V8 selective medium. Sand was the most effective substrate for removing zoospores; no zoospores were detected in water passed through 40 and 60 cm of sand. The other four substrates were less effective at physically filtering zoospores. Zoospore densities in effluent were reduced significantly by clay and crushed brick, but zoospores were not completely eliminated at any depth tested. The effect of adding a biological component to each substrate will be examined using runoff water from an ornamental plant nursery. Runoff will be continuously pumped through columns until a natural microbial film is established within each substrate. Zoospore removal will be tested before and after microbial growth develops to evaluate biological filtration efficiency. Sand, crushed brick, and calcined clay will be examined at a 10-cm depth and Kaldnes medium and polyethylene beads will be examined at a 60-cm depth, based on results from the physical filtration experiment. In the future, selected substrates will be evaluated for removal of pathogen propagules in the field using subsurface-flow constructed wetlands at a production nursery. If effective, use of constructed wetlands at nurseries could provide an ecological alternative to pathogen mitigation by reducing reliance on agrichemicals and should encourage use of recycled water.

**MP220 RME: Exploring the Upper Bounds of Upper-bound Exposure Parameters in Deterministic Human Health Risk Assessments** D. Chin, P. Anderson, B. Magee, ARCADIS. United States Environmental Protection

Agency (USEPA) human health risk assessment guidance defines a reasonable maximum exposure (RME) scenario as "the highest exposure that is reasonably expected to occur at a site," provided that both quantitative data and professional judgment indicate that the exposure does not exceed the range of possible exposures. High end exposure parameters, chosen "to be conservative" without regard for the core purpose of the RME, result in unrealistic estimates of risk that contribute little to the evaluation of a site. Three considerations concerning the development of realistic RME scenarios are presented here. First, USEPA guidance does not support choosing more than one or two high end exposure parameters for a single RME scenario, particularly if the scenario involves more than one exposure pathway. Next, specific exposure parameters developed for an RME scenario should not be confused with those developed for bounding estimates of risk. Finally, the ability of a site to sustain the chronic high end contact rates used in an RME scenario is an appropriate and necessary consideration for a meaningful human health risk assessment. Examples based on recent, real-world risk assessments will be provided to demonstrate each consideration.

**MP221 The Chemical Footprint: An Emerging Indicator of Environmental Performance** K. Hitchcock, ChemRisk, Associate Health Scientist; J. Panko, P. Scott, ChemRisk. Currently, companies report ecological, carbon, and water footprints to communicate their environmental performance to the public. However, a measure of the impact of the chemicals contained in a product or used in its manufacture, the chemical footprint, will likely become increasingly common in the coming years. This footprint provides manufacturers with a comprehensive evaluation of the chemicals used and produced for a given consumer or industrial product. The chemical footprint includes an inventory of the chemicals as well as an evaluation of its positive and negative environmental characteristics. Characteristics of a chemical that may be evaluated include its identification as a known carcinogen, its persistence, its bioaccumulation potential, its mobility, and its exposure potential. Armed with this knowledge, companies are able to identify areas for improvement and safeguard against current and future regulatory compliance issues. Using a popular shampoo as an example, it is clear to see how a product-level chemical footprint, with a narrow scope, can be evaluated and communicated to the consumer. However, it is also easy to see how it can be extended to the entire manufacturing process for a more complete picture. The chemical footprint represents a new measurement of environmental performance that evaluates chemical properties, such as intrinsic hazards and volume used, which are not typically accounted for by other measures of environmental performance.

**MP222 The First International Environmental Decision by The World Court on Paper Mill Impacts** T. Deardorff, Exponent, Senior Managing Scientist; C. Menzie, J. Ma, J. Salatas, T. Wickwire, M. McArdle, Exponent; D. Pryke, Alliance for Environmental Technology. The World Court in The Hague, a court that adjudicates disputes between nations, recently ruled on a landmark environmental dispute between Uruguay and Argentina regarding the construction of a world-class pulp mill on Uruguayan side of the Uruguay River. There were two primary Argentine challenges against the development in Uruguay. The first challenge alleged that the Orion mill was not a state-of-the-art mill because it did not use best available technology (BAT) and would not be among the most environmentally efficient in the world. We compared the actual performance of the mill with international new source performance standards and other state-of-the-art, modern and recently commissioned mills in the world to demonstrate that the mill met these world class standards. Argentina's second challenge was that the mill would adversely impact the adjacent aquatic environment because the mill did not use tertiary treatment and, thus, could not obtain world class standards for nitrogen and phosphorus releases, citing a recent and massive bloom of cyanobacteria in the adjacent river as evidence of their assertion. We demonstrated, using satellite imagery of the river before and after the bloom, that the origins of the bloom were not associated with mill activities. In a 13-4 decision, the World Court ruled that there was "no conclusive evidence in the record to show that Uruguay has not acted with the requisite degree of due diligence or that the discharges of effluent from the Orion (Botnia) mill have had deleterious effects or caused harm to living resources or to the quality of the water or the ecological balance of the river since it started its operations in November 2007." The court went on to say that the Court's judgment vindicated Uruguay's assurances that, based on a comprehensive environmental impact analysis and strict monitoring of the mill's discharges, it was environmentally sound in all respects with no risk of harm to the

river or its surroundings. The court's decision on the environmental issues represented the first definitive decision in a case involving international environmental law.

**MP223 Use of Aquatic Macrophyte Combinations to Mitigate Aqueous Concentrations and Effects of Permethrin and Nitrogen** R.E. Lizotte, USDA Agricultural Research Service, National Sedimentation Laboratory, USDA-ARS National Sedimentation Lab, Water Quality and Ecology Research Unit, USDA-ARS National Sedimentation Lab, Water Quality and Ecological Processes Unit; M.T. Moore, USDA-ARS, National Sedimentation Laboratory, USDA-ARS National Sedimentation Laboratory, National Sedimentation Laboratory. Aquatic vegetation occurring in wetland habitats can be managed to mitigate agricultural contaminants exiting row-crop fields during storm events. Most such vegetation occurs as a mixture of different species and there is a need to better understand how multiple species can be managed to improve mitigation efficiency. In this study, combinations of two aquatic macrophytes, parrot feather (*Myriophyllum aquaticum*) and cattail (*Typha latifolia*), were used to assess mitigation of aqueous concentrations and effects on permethrin and nitrogen. Using hydraulically connected wetland microcosms (~50 L) in series, both species were tested singly (upstream microcosms *Myriophyllum* only, M; *Typha* only, T) and in combination (upstream to downstream *Myriophyllum* into *Myriophyllum*, MM; *Typha* into *Myriophyllum*, TM) to examine the influence of varying species along a linear hydraulic flow path. Upstream microcosms were dosed with a mixture of permethrin and nitrogen (as ammonium nitrate) simulating agricultural runoff. Aqueous concentrations of permethrin, NH<sub>4</sub>-N, and NO<sub>3</sub>-N were measured at 0 h (pre-treatment) through 8 h and again at 24, 48, 72, 96, and 168 h. Effects were assessed using *Hyalella azteca* 48 h aqueous laboratory bioassays at 0, 4, 24, and 48 h. Results showed permethrin was rapidly removed from the water column within 24 h. Mean permethrin half-lives (t<sub>1/2</sub>) ranged from 3-7 h with TM having a significantly ( $p < 0.05$ ) longer t<sub>1/2</sub> (6.8 h) than other treatments. Permethrin t<sub>1/2</sub> was correlated ( $r = -0.5256$ ,  $p < 0.05$ ) with overall macrophyte biomass. Nitrogen as NH<sub>4</sub>-N and NO<sub>3</sub>-N steadily decreased over the 168 h study periods. Mean nitrogen t<sub>1/2</sub> ranged from 59-238 h and 72-150 h for NH<sub>4</sub>-N and NO<sub>3</sub>-N, respectively, with T most rapid and M the least. Nitrogen t<sub>1/2</sub> was also correlated (NH<sub>4</sub>-N:  $r = -0.8649$ ,  $p < 0.01$ ; NO<sub>3</sub>-N:  $r = -0.7859$ ,  $p < 0.01$ ) with overall macrophyte biomass. Effects of permethrin and nitrogen mixture toxicity occurred within 4 h and were mitigated within 24 h. Using the toxic unit model approach, the primary source of observed toxicity was permethrin. Despite apparent differences by aquatic macrophyte type, observed differences were due to overall differences in macrophyte biomass. As a result, overall macrophyte biomass appears to be the most important constituent in mitigating permethrin and nitrogen.

**MP224 Using a Multiple Line of Evidence Approach for Determining a Remedial Footprint for Sediment** M. Pattanayek, B. DeShields, ARCADIS. Sediment is composed of a complex matrix of soils, sand, organic matter, or minerals that accumulate on the bottom of a water body, which makes data interpretation difficult. Measured concentrations of chemicals in sediment do not address toxic effects or distinguish the bioavailable fraction from the bound fraction (which is what is measured in the laboratory results). To determine "impacted" locations from and ecological risk perspective, sediment quality assessments are often supported by other lines of evidence (LOEs) such as toxicity tests/bioassays and benthic community analyses. None of the individual LOEs are considered sufficient for assessing sediment quality impacts resulting from potentially toxic chemicals. A given LOE may overestimate or underestimate risk to a benthic community and may not address causality of specific chemicals. For remedial decision-making, sediment quality assessment (bioassay, benthic community, etc.) measures are often combined into a multiple line of evidence (MLOE) triad to integrate exposure and effects. We applied an MLOE approach at an estuarine site in California to categorize locations as "impacted" or "unimpacted" and estimate the remedial footprint for a mudflat area subject to metals and polychlorinated biphenyl (PCB) contaminated runoff from an adjacent upland site. The MLOE approach used for assessing the intertidal mudflat area was similar to the California Sediment Quality Objectives (SQO) triad approach but also included other LOEs such as identification of risk drivers based on an ecological risk assessment, hotspot analyses, and best professional judgment to provide a more robust assessment of sediment quality. Sediment conditions were categorized as "impacted" for a location if more than one LOE (other than chemical concentration) indicated potential

concern, or based on best professional judgment. If only one LOE was present without any other supporting evidence, risk drivers were considered and best professional judgment was used to determine if the location could be categorized as "unimpacted." Using the MLOE approach, impacted sediment was delineated horizontally. Vertical delineation was also conducted; however, only limited LOEs were applicable. The MLOE approach used for the impacted mudflat was also compared to the SQO triad approach.

**MP225 Using Statistical and Bioavailability Analyses to Identify Benthic Organism Risk Drivers** A.B. Francisco, M.K. Butcher, M.B. Certo, J.J. Gravenmier, ARCADIS. Identification of risk drivers for benthic organism exposure to impacted sediment is frequently complicated by complex constituent of potential concern (COPC) mixtures. We present a methodology using toxicity tests, statistical analyses, and bioavailability assessments of associated sediment analytical data to narrow down an initial list of 33 COPCs to two likely risk drivers. We identified 33 initial COPCs by comparing sediment analytical chemistry data to conservative screening values. We then identified potential risk drivers by performing a ranked regression analysis of *Eohaustorius estuarius* 10-day acute toxicity test results against sediment analytical data. In our regression analysis, we evaluated bulk concentrations of metal COPCs, bulk and organic carbon normalized concentrations of non-polar organic COPCs, and sulfide and total organic carbon concentrations. The ranked regression analysis identified five distinct COPCs whose correlations were statistically significant at a p-value of 0.05. We then identified the likely risk drivers by qualitatively and quantitatively evaluating bioavailability. Qualitative evaluations of sediment chemistry indicated that divalent metal COPCs identified in the regression analysis (i.e., copper and lead) were unlikely to be risk drivers due to anoxic conditions and high sulfide concentrations (up to 17,200 mg/kg). These conditions would likely result in divalent metals binding with available sulfides to create insoluble metal-sulfide complexes that are largely not bioavailable and unlikely to cause toxicity. We also conducted quantitative bioavailability evaluations following United States Environmental Protection Agency equilibrium partitioning sediment benchmark (ESB) guidance. Quantitative evaluations indicated that DDT and metabolites (DDTr), chlordane, and endosulfan and metabolites exceeded their respective ESBs. Further evaluation of statistical trends and analytical data indicated that endosulfan and metabolites toxicity correlation and ESB exceedances were an artifact of elevated detection limits resulting from elevated concentrations of DDTr and chlordane. Therefore, we identified DDTr and chlordane as likely drivers of benthic toxicity, and are now able to more effectively make future risk management decisions.

**MP226 Environmental Sustainability Innovations and Coastal Infrastructure Design** T.J. Fredette, US Army Corps of Engineers; B.C. Suedel, US Army Engineer Research and Development Center, CEERD-EP-R, US Army Engineer Research and Development Center, Environmental Laboratory, Waterways Experiment Station EP-R; C.J. Banks, US Army Engineer Research and Development Center, CEERD-EP-R, US Army Engineer Research and Development Center, Research Biologist; S. Brasfield, US Army Engineer Research and Development Center, Environmental Laboratory. Since the advent of the National Environmental Policy Act (1969), the design of coastal structures such as breakwaters, jetties, and seawalls in the United States have focused on balancing engineering and environmental goals, with great emphasis being given to minimizing unacceptable adverse environmental impacts. More recently, the concept of environmental sustainability has begun to influence the way in which planners and designers approach projects. In such instances, designers have sought ways to move beyond the somewhat narrow minimize-impact focus to incorporate features into projects with the explicit intent of improving the environmental compatibility of the infrastructure. In 2009, the US Army Corps of Engineers (USACE) began an initiative to identify examples and opportunities where application of this concept has already been employed or might be possible. Through these investigations, a number of specific projects were identified that include (1) the creation of tern nesting habitat on Great Lakes breakwaters, (2) the notching of Missouri River rock chevrons to create a more diverse pool and island habitat, (3) the placement of pea gravel aprons at the base of breakwaters to provide fish spawning habitat and decrease wave energy, and (4) the creation of spurs off of a shoreline revetment to develop habitat complexity. The USACE investigation also identified a wide range of potential ideas that could be incorporated into designs in marine, lacustrine, and riverine environments. These included the incorporation of specifically

designed reef modules into structures in place of normal construction materials, the addition of tide pool elements into seawalls or breakwaters, creation of marine mammal haul-out shelves, and others. Ongoing work consists of identifying other existing sites or potential pilot projects where evaluation of benefits can be documented.

**MP227 Estimating Time-to-Compliance for Remediation Options Based on Monitored Natural Attenuation** S. Selden, P.E. Goodrum, Environmental Resource Management. Reliable estimates of time-to-compliance are needed to evaluate monitored natural attenuation as a viable remediation option. Most often, simple kinetic models (e.g., first-order “exponential decay”) are developed to predict the rate of change in concentrations over time and/or space. These models are mathematically convenient and supported by regulatory guidance and convention. However, too often model selection is viewed as purely a goodness-of-fit exercise without considering the conceptual site model or the strengths and limitations of alternative approaches. A decision process is presented to encourage consideration of a broader range of models and exploratory data analysis steps. Examples with groundwater data are provided to illustrate how the decision process can guide in the selection of an appropriate model given site conditions, including (1) assumption that concentrations approach background rather than zero; (2) influence of non-detects on parametric and non-parametric methods; (3) corrections for data transformation bias; (4) influence of potential outliers; and (5) determination of representativeness of historical data. While the decision process is illustrated for investigations involving compliance monitoring for groundwater, the approaches are generally applicable to time series analysis for a wide range of exposure media and remedial action objectives.

**MP228 Evaluating CO<sub>2</sub> Emission from Automobiles Through Employing Environmental Housekeeping Book Method and Trip Demand Estimation Model** N. Kojima, Osaka Univ; S. Yao, Osaka Univ; K. Nakazawa, Osaka Univ, Graduate School of Engineering; A. Tokai, Osaka Univ. We have been developing new risk evaluation method named ‘Risk Durability Method’. This method includes the view point of risk-risk tradeoff and value of information analysis. We have tried to evaluate the risk durability which includes risk tradeoff and Value Of Information (VOI) viewpoints through automobile industry environmental system as a case study. This method includes the policy or development trend of industry in scenario making level, so at that time, Risk Durability Method is depend on development of science technology. However, in order to manage several risk tradeoff in certain level, consumers who enjoyed technology should consider various convenience in their life and environmental burden. To understand the consumer’s needs to automobiles under the environmental constraints, we employ the environmental housekeeping book method. In this study, we applied this method to public citizen and estimated the amount of CO<sub>2</sub> emission from car exhaust gas we could cut. Preliminary survey of environmental housekeeping book method on car usage was conducted to environmental volunteers who lives in Suita city, Osaka, Japan in January 2011. We collected 72 samples out of 189 questionnaires distributed to potential interest groups. The questionnaire of environmental housekeeping book consists of one week diary form and some relevant questions on everyday car usage and life activities. Through this survey, we found that some of respondents are willing to change the way of car usage by checking their everyday life activities through this environmental housekeeping method. As one of the potential operational variables, for example, they tried to walk or use bicycle instead of cars to commute and buy groceries. In this sense, the frequency and period of using cars could be controlled. They also chose options of public transportation, bicycle, walking, and a rental car instead of their own automobiles. These results can be integrated with the trip demand estimation model which we applied to Kinki region including Osaka, Japan. Finally we estimated the amount of potential reduction of CO<sub>2</sub> emission and some other environmental loads from automobiles, and discussed their tradeoffs.

**MP229 Exposure Assessment for Antimicrobial Copper Use in Building Materials** M. Velleux, HDR|HydroQual; A. Redman, HydroQual; P. Paquin, R. Santore, HDR|HydroQual. Copper releases from antimicrobial agents used in building materials such as algae-resistant shingles and treated wood may pose risks to aquatic and terrestrial life. Exposure assessments for antimicrobial copper in urban areas are complicated because copper-containing building materials may be present in roofs, decks, fences, and utility poles that are installed in both densely clustered and widely dispersed

locations. To address the need for a more refined exposure assessment approach, the TREX (Two-dimensional Runoff Erosion and Export) watershed model was used to estimate antimicrobial copper exposures in surface water, soil, and sediment from building materials in a simulated urbanized setting. TREX differs from other runoff models in that it has the capability to represent the land surface with a very high level of spatial detail. A 21.4 km<sup>2</sup> watershed was used as the urban setting and was simulated at a 90-m resolution with 2,558 grid cells. The stream network within the watershed was 23 km in length and was represented by 258 grid cells. Each cell can be uniquely characterized with respect to land surface elevations, soil properties, rainfall-runoff, chemical release rates, and other properties. Copper release rates from building materials were determined based on surface area and rainfall rates. Benchmark effects levels for copper in surface water were determined using the Biotic Ligand Model (BLM) and regional water quality conditions. Benchmarks for copper in soil and sediment were determined using experimentally-derived toxicity endpoints and site-specific soil chemistry as well as equilibrium sediment guidelines. Simulations were performed for a 10-year period with 772 storms defined using 15-minute rainfall data. Simulated copper exposures from algae-resistant shingles were relatively low and did not exceed effects levels in any media. Simulated copper exposures for treated wood were larger than those for shingles. Exposures in soil and sediment did not exceed benchmarks. Treated wood copper exposures exceeded water quality benchmarks twice during the 10-year simulation period. Given the conservative nature of the assessment, these results suggest that potential risks posed by releases of antimicrobial copper used in building products are low.

**MP230 Hexabromocyclododecane (HBCDD) – A Brominated Flame Retardant of Very High Concern in REACH Regulation** A.A. Jensen, Nordic Institute of Product Sustainability, Environmental Chemistry and Toxicology; A. Bergman, Stockholm Univ. Hexabromocyclododecane (HBCDD or HBCD) is used mainly as a flame retardant in polystyrene-based insulation products and in textile products. It has a complex chemistry, and of particular concern is the capacity of this lipophilic and persistent organic pollutant to accumulate in the food chain, leading to progressively increasing levels in human tissues and in wildlife. The extent of HBCDD accumulation in the environment, in wildlife and humans correlates directly with its ever-more prevalent use. Despite this alarming trend, only limited toxicological information is available to assess its long-term implications for health or the environment. Nevertheless, HBCDD continues to be used and that despite the availability of alternatives. The European Chemicals Agency did in 2008 identify HBCDD as 1 of 14 substances of “Very High Concern”, and in September 2010 HBCDD was added to REACH’s Authorization List. In February 2011 HBCDD was selected to be phased out in 3-5 years by EU REACH Regulation. In October 2010 a committee under the Stockholm Convention has assessed the risks from HBCDD and concluded that HBCDD fulfills the criteria of a persistent organic pollutant (POP), and the committee recommended a global ban of HBCDD use. This presentation provides a succinct up-to date overview of HBCDD’s properties and discusses the risks associated with its prevalence in our homes and immediate environment. HBCDD was also included in the “San Antonio Statement on Brominated and Chlorinated Flame Retardants” signed by 245 scientists from 22 countries in September 2010.

**MP231 Lab Scale Demonstration of an Electrode Enhanced Cap to Encourage Hydrocarbon Degradation** F. Yan, The Univ of Texas at Austin. Capping is typically used to control contaminant release from the underlying sediments. However, the presence of conventional sediment caps will eliminate or slow natural degradation processes that might otherwise occur at the surface sediment. The objective of this study was to explore the potential of a novel reactive capping method-electrode enhanced cap for the remediation of contaminated sediment. Carbon cloth electrodes were placed in thin sand capping layer to remediate PAH contaminated sediments in a laboratory microcosm. A small voltage, i.e., 2V, was applied to create an oxidative environment in favor of PAH degradation at the cap-sediment interface. Oxidation and Reduction Potential (ORP) profiles showed redox potential approximately 100 mV higher than in controls could be sustained at the cap-sediment interface. Redox sensitive species (i.e., oxygen, Fe(II), Mn(II) and S(II)) were quantified by voltammetric measurement. The profiles of redox sensitive species corresponded well with the ORP measurement. Porewater concentration of PAH was obtained by Solid Phase Micro Extraction (SPME), and it provided evidence of PAH degradation



under electrode enhanced caps. qPCR analysis for PAH degrading genes showed that PAH degrading bacteria were stimulated by the production of oxygen. No significant degradation of the low cost carbon cloth materials was observed during the course of the experiment. Potentially, this novel electro-reactive capping can provide an inexpensive and long term technique to manage the contaminated sediment.

**MP232 Phytoremediation of Atrazine from Simulated Surface Water Runoff Using Switchgrass** V.C. Albright, I.J. Murphy, J. Anderson, J.R. Coats, Iowa State Univ, Entomology. Atrazine is one of the most widely used herbicides in the agricultural industry today and, as a result, can commonly be found in many bodies of water in the Midwest. Atrazine and its metabolites have been shown to have many adverse effects on plants and animals within an ecosystem. Previous research has shown that switchgrass can take up and degrade atrazine into its less toxic metabolites. Greenhouse studies have shown that mature switchgrass plants take up atrazine and quickly metabolize it and dispose of it. The current research project has two objectives: (1) determine degradation of atrazine using radiolabeled  $^{14}\text{C}$ -atrazine to allow for more sensitivity than the previous studies which used gas chromatography and (2) determine the possible exudation of atrazine metabolites from the switchgrass after uptake and degradation.

**MP233 Scenario Analysis of Alternative Fuels of Automobile Based on the Concept of Risk-Durability** Y. Higuchi, N. Wada, K. Mochizuki, A. Tokai, Osaka Univ, Division of Sustainable Energy and Environmental Engineering, Graduate School of Engineering. We have been studying new risk evaluation method which includes two viewpoints, that is, Value Of Information and risk-risk tradeoff analysis. We named this new risk evaluation method "Risk Durability Method" and have conducted a case study regarding the fuel switch of automobiles. We focused on the environmental system of automobile industry and picked up cases of automobile use, because automobiles enable us to improve the mobility in everyday life activities. Followings are points of the argument of this research based on our analysis. The first point is the emission profiles of environmental load from automobiles through their life stages in connection with multiple risks. We estimated the emission profile of carbon dioxide ( $\text{CO}_2$ ) and other types of environmental loads, and their tradeoffs. The second point is the risk profile of switching fuels of automobiles. Here, the risk profile means the tradeoff relationships between different kinds of risk indexes: health risk, ecological risk, global risk and resources conservation risk. We estimated these risks at the specific time cross-sections, and clarified the risk profile and their risk-risk tradeoffs. Margin of exposure is employed for human and ecological health effects,  $\text{CO}_2$  is for global warming and Total Material Requirement is for resources conservation risk index. Then we examine risk tradeoffs using health, eco, resources, and low  $\text{CO}_2$  risk indexes of two time cross-sections. In addition, we will examine the suitable scenario where we can control these four risks. Finally we discuss the results from the viewpoint of risk durability of the system of automobile including consumers and their usage.

**MP234 Successful Application of Thin Layer Placement in Two Dynamic Urban Water Bodies with Heavy Human Disturbance** J.A. Colton, King County Dept of Natural Resources, Water and Land Resources Division, King Cty. Dept of Nat. Resources, Water and Land Resources Division; J.H. Stern, King County, Dept of Natural Resources. King County has conducted sediment remediation at two different sites (Pier 53/55 and Duwamish Diagonal in Seattle, WA) where multiple remediation technologies were implemented. In 1992, a 3-foot sediment cap and 1-foot sand layer (thin layer placement) were placed on 4.5 acres, offshore of Piers 53 – 55 on Seattle's downtown waterfront. Long-term monitoring at this site included periodic sediment chemistry sampling by core and grab in the experimental thin layer placement (10-18 inches depth) and the 3-foot cap to study enhanced natural recovery (ENR), and monitoring for stability and benthic community composition over a 10 year monitoring program. In 2004, a 7-acre area of contaminated sediment at Duwamish Diagonal in Seattle's Lower Duwamish Waterway was remediated by mechanical dredging to an average depth of 5 feet and covered with an engineered cap. Post-cap monitoring indicated dredging residuals had accumulated in the immediately adjacent area and significantly increased polychlorinated biphenyls (PCB) concentrations in the surface sediments. A thin layer of clean sand (6-9 inches depth) was placed across 4 acres, over the highest PCB concentrations, to enhance natural attenuation and immediately reduce exposure. The remaining area was projected to naturally attenuate to pre-dredge

concentrations within 2 to 3 years. Long-term monitoring at this site included surface sediment chemistry grab samples collected annually for at least 5 years across the areas covered by engineered cap and a thin layer of sand, as well as in the perimeter where natural remediation was expected. These two projects provide a unique opportunity to compare the performance of thin layer placements at different thicknesses to each other, and to two other remediation alternatives (i.e., engineered caps and monitored natural remediation). Although thin layer placements are intended to accelerate natural remediation processes, at both sites, the thin layer placements performed similarly to engineered caps, showing little to no signs of mixing by biota at the contaminated sediment/sand interface. The benthic community monitoring at Pier 53/55 also suggested the thin sand layer smothered the benthic community, as occurred with the engineered cap, and the recolonization rates were the same. Both the ENR and engineered capping technologies successfully isolated contaminants and remained stable throughout the long-term monitoring program. Monitored natural remediation recovered to pre-dredge chemistry faster than predicted.

**MP235 Uncertainty Analysis of Environmental Industry System Focused on the Usage of DecaBDE** K. Nakazawa, Osaka Univ, Graduate School of Engineering; A. Tokai, Osaka Univ. Chemical risk evaluation has been conducting to concentrate each chemical which we concern. As a result, these assessments were used for decision making for policy or/and self regulation for industries. However, there are two issues which we need to argue more. Firstly, the chemical risk evaluation need to shift to comprehensive risk assessment considering about the some kinds of uncertainty. It contains various risks derived from certain industrial products, because each chemical was usually used for many kinds of products. Namely, we need to add the view point of risk-risk tradeoffs between environmental risks in risk evaluation for substance and products. Secondly, risk evaluation has been conducting using the data which are available at that time. Therefore, the viewpoint of information sufficiency level for the purpose of risk reduction, in other words, Value Of Information (VOI) should be examined for the clarification of them. We tackled to construct the new risk evaluation method based on the concept 'Risk Durability Method' which includes risk tradeoff and VOI viewpoint, through case study. We have conducted the case study focused on one of brominated flame retardant, decabromodiphenyl ether and evaluated health risks for human health in two time cross-sections with the viewpoint of VOI. We applied substance flow analysis method to DecaBDE and clarify the profile of exposure pathway. Firstly, we carried out sensitivity analysis and clarify the high priority datasets for risk evaluation, then we discussed the variability and uncertainty of data regarding the estimated risk level. In this presentation, we will show the evaluated results of the uncertainty of decabromodiphenyl ether health risk. Through literature survey and delphi type expert questionnaire survey study, we identify the uncertain parameters. Then using software tool of uncertainty analysis, we evaluate it using three indexes of Expected Value of Perfect Information (EVPI), Expected Value of Sample Information (EVSII), and Expected Value Including Uncertainty (EVIU).

**MP236 Zebrafish as an Alternative Model for the Quantitative Assessment of Chemical-induced Developmental Toxicity in Humans** C.R. Fleming, Oak Ridge Institute for Science and Education; J.C. Lambert, United States Environmental Protection Agency, National Center for Environmental Assessment. There is increasing interest in the use of alternative animal models in place of traditional rodent bioassays for characterization of chemical toxicity. Benefits of the zebrafish model include their small size, low cost of maintenance, rapid breeding cycle, large number of offspring, and transparent early life stages. Several analyses have demonstrated the utility of zebrafish as a predictive screening tool for mammalian developmental toxicity and teratogenicity. However, for human health risk assessment purposes, alternative toxicity testing models should inform quantitative dose-response, not simply provide an identification of hazard. To begin to examine the feasibility of applying quantitative dose-response data from zebrafish embryonic bioassays to human health risk assessment of potential developmental toxicants, we identified dose-response datasets in zebrafish and rodents for several developmental toxicants with differing toxicokinetic properties. Zebrafish studies were excluded from our analysis if aqueous exposure concentrations were not accompanied by an estimate of the dose to embryos. Reported doses in the fish and rodent studies were adjusted to a common dose metric and effect levels were compared between species to determine if dose-response data from assays using zebrafish embryos

can be considered as predictive of human developmental toxicity as more conventional mammalian model species. Preliminary results based on developmental NOAELs and LOAELs for a subset of chemicals showed good concordance of the dose-response data between rodents and zebrafish, indicating that it may be feasible to design a zebrafish assay that could be used to inform human health risk assessment without the introduction of a high degree of uncertainty. Zebrafish assays with appropriate dosing methods are needed for a larger database of chemicals to verify these findings. If zebrafish can be validated as a model organism for human developmental toxicity, assessment data gaps could be filled in a time- and cost-efficient manner. Thus, this work could have a significant impact on human health risk assessment of chemicals which lack developmental data. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the USEPA.

**MP237 Anniston AL PCB Site: 1. Relative Endpoint Sensitivity of *H. azteca*, *C. dilutus*, and Mussel (*L. siliquioidea*) Chronic Sediment Toxicity Tests** J.K. Stanley, US Army Engineer Research and Development Center, Environmental Laboratory; N.E. Kemble, US Geological Survey, Biological Resources Division, Columbia Environmental Research Center; J. Farrar, US Army Engineer Research and Development Center; J.L. Kunz, USGS; J.G. Sims, US Army Engineer Research and Development Center, Environmental Lab (EP-R); J.F. Williams, US Army Engineer Research and Development Center, Environmental Laboratory; P. Chappell, Badger Technical Services; J.A. Steevens, US Army Engineer Research and Development Center, Waterways Experiment Station; C.G. Ingersoll, USGS, Columbia Environmental Research Center; M.S. Greenberg, USEPA Environmental Response Team; S.R. Thoms, USEPA, Region 4, Superfund Division, Superfund Support Branch, USEPA, Region 4, Waste Division, Office of Technical Serv. Polychlorinated biphenyls (PCBs) in the Anniston, AL, USA area were released from the operations of the former Monsanto Corporation's PCB manufacturing plant resulting in spatially expansive sediment contamination by PCBs in Choccolocco Creek and its floodplain. An integrated, multi-agency research team was assembled to assess bioavailability and toxicity of PCBs in sediments collected from the site in August 2010. As part of this investigation, a total of 32 sediment samples were collected from selected locations within the study area to support chemical characterization and whole-sediment toxicity or bioaccumulation testing. Of these, up to 26 sediment samples were evaluated in chronic toxicity tests (up to 20 samples from the site and six samples from a reference area), based on the measured concentrations of total PCBs and total organic carbon (i.e., to provide a broad gradient of total PCB concentrations in sediment). *Hyalella azteca* (HA) 42-d, mussel (*Lampsilis siliquioidea*; LV) 28-d and *Chironomus dilutus* (CD) life-cycle testing was performed in basic accordance with ASTM and EPA guidance. While data analysis and interpretation are ongoing, initial results indicate that sensitivity of chronic endpoints based on organic carbon normalized sediment PCB concentrations for the three organisms used follow the pattern: (Least Sensitive) CD survival < HA survival = LV survival < LV weight = HA weight < CD 13-d AFDW = CD 13-d biomass = LV biomass < CD emergence = HA young per female < CD egg cases per replicate = CD eggs per replicate (Most Sensitive). These initial results will be supplemented with additional data including other endpoints assessed in order to fully assess relative endpoint sensitivity in PCB contaminated sediment exposures using field-collected sediments from this system.

**MP238 Anniston AL PCB Site: 2. Inter-laboratory Chronic Sediment Toxicity Testing with Amphipods (*Hyalella azteca*) and Midge (*Chironomus dilutus*)** N.E. Kemble, US Geological Survey, Columbia Environmental Research Center, USGS – Biological Resources Division, Columbia Environmental Research Center; C.G. Ingersoll, USGS, Columbia Environmental Research Center; J.L. Kunz, USGS; D.D. Farrar, J.K. Stanley, J.A. Steevens, USACE; D.M. MacDonald, MESL; M.S. Greenberg, USEPA Environmental Response Team; S. Thoms, USEPA. Polychlorinated biphenyls (PCBs) in the Anniston, AL, USA area were released from the operations of the former Monsanto Corporation's PCB manufacturing plant resulting in spatially expansive sediment contamination by PCBs in Choccolocco Creek and its floodplain. An integrated, multi-agency research team was assembled to assess bioavailability and toxicity of PCBs in sediments collected from the site in August 2010. As part of this investigation, a total of 32 sediment samples were collected from selected locations within the study area to support chemical characterization and whole-sediment toxicity or bioaccumulation testing. Given difficulty for one laboratory to

concurrently test multiple sediment samples in chronic sediment toxicity tests with midge and amphipods, sediments were split between two toxicity testing laboratories (US Army Corp of Engineers, USACE) US Geological Survey; USGS). Initial inter-laboratory comparisons of the life-cycle midge and 42-d amphipod whole-sediment toxicity tests were conducted with six samples selected to represent a broad PCB gradient. Toxicity tests were conducted following ASTM and EPA guidance and overlying test water was selected to represent site conditions and was relatively similar at both laboratories. Both laboratories met ASTM and EPA test acceptability requirements for both species (control survival was >94%). Similar decreases in survival, growth, emergence, or reproduction of midge or amphipods was observed across the six sediments evaluated in inter-laboratory testing and across an additional six samples evaluated in intra-laboratory testing of midge by USGS or in intra-laboratory testing of amphipods by USACE. Normalizing responses of test organisms to control or reference sediment helped reduce inter-laboratory variability in reproduction endpoints for both species. However, growth of amphipods tended to be greater in sediments tested by the USGS compared to USACE. Results of this study indicate there will likely be minimal inter-laboratory bias in subsequent toxicity testing of site sediments with midge by the USGS and with amphipods by USACE.

**MP239 Anniston AL PCB Site: 4. Solid Phase Micro Extraction (SPME) Fibers Predict Bioavailability and Bioaccumulation of PCB from Sediment** J.A. Steevens, US Army Engineer Research & Development Center, Waterways Experiment Station; J. Stanley, US Army ERDC; N. Kemble, USGS; D. Farrar, G. Lotufo, US Army ERDC; J. Kunz, USGS; J. Sims, J. Williams, P. Chappell, US Army ERDC; M. Greenberg, EPA; C. Ingersoll, USGS. Polychlorinated biphenyls (PCBs) in the Anniston, AL, USA area were released from the operations of the former Monsanto Corporation's PCB manufacturing plant resulting in spatially expansive sediment contamination by PCBs in Choccolocco Creek and its floodplain. An integrated, multi-agency research team was assembled to assess bioavailability and toxicity of PCBs in sediments collected from the site in August 2010. As part of this investigation, a total of 32 sediment samples were collected from selected locations within the study area to support chemical characterization and whole-sediment toxicity or bioaccumulation testing. The bioavailability and bioaccumulation of PCB from site sediments was investigated using passive solid phase micro extraction (SPME) samplers and the 28-day *Lumbriculus variegatus* whole-sediment bioaccumulation tests. Passive samplers were placed in laboratory toxicity and bioaccumulation test chambers for up to 28 days to measure the bioavailable pore water fraction of PCBs. Variability of the PCB concentrations in the pore water fraction, measured using the SPME was low between laboratories and among toxicity and bioaccumulation test chambers. The *L. variegatus* exposures were conducted for 28 days and tissues analyzed for total lipid and PCB congeners and homologs. A strong relationship was observed for SPME concentrations and lipid normalized PCB concentrations in oligochaetes ( $r^2 = 0.99$ ). Similarly, a strong relationship ( $r^2 = 0.91$ ) between lipid normalized PCB concentrations in tissue and organic carbon PCB concentrations in sediment was observed. In the overall assessment of toxicity and bioaccumulation at this site, these measures of bioavailability and bioaccumulation will provide strong evidence about the relative availability of PCB to aquatic organisms and potential for bioaccumulation in a food web risk assessment.

**MP240 Anniston AL PCB Site: 5. Relationships Between Sediment Chemistry and Sediment Toxicity** D.M. MacDonald, MESL; C.G. Ingersoll, USGS, Columbia Environmental Research Center; W.G. Brumbaugh, N.E. Kemble, J.L. Kunz, USGS; J. Steevens, J.K. Stanley, F. Daniel, USACE; S. Thoms, M.S. Greenberg, USEPA. The Anniston PCB Site is located in north eastern Alabama USA. Environmental concerns focus primarily on polychlorinated biphenyls (PCBs) released from 1935 to 1971 from production waste, spills, effluent discharges, releases from landfills, and storm water runoff. USEPA, Solutia/Pharmacia, and Dept of Interior are evaluating risks to ecological receptors associated with exposure to PCBs and other chemicals of potential concern (COPCs) in environmental media at the site. As part of this investigation, a total of 32 sediment samples were collected from the study area by Solutia/Pharmacia to support chemical characterization and whole-sediment toxicity and bioaccumulation testing needed to support a CERCLA RI/FS. Of these, up to 26 samples were selected for toxicity testing and 15 samples were selected for bioaccumulation testing, based on concentrations of total PCBs and total organic carbon (i.e., to provide a broad gradient of total PCB concentrations in

sediments tested). Two toxicity tests were conducted including life-cycle whole-sediment toxicity tests with the midge, *Chironomus dilutus* (Endpoints: survival, growth, biomass, emergence, and reproduction) and a 42-d whole-sediment toxicity tests with the amphipod, *Hyalella azteca* (Endpoints: survival, growth, biomass, and reproduction). Bioaccumulation of PCBs was evaluated in 28-d whole-sediment exposures with the oligochaete, *Lumbriculus variegatus*. The Dept of the Interior evaluated splits of samples in 28-d whole-sediment toxicity tests conducted with the mussel, *Lampsilis siliguoidea* (Endpoints: survival, growth, and biomass). Peepers and acid-volatile sulphide were used to estimate bioavailability of metals and solid-phase micro extraction (SPMEs) were used to estimate bioavailability of PCBs. Matching sediment-chemistry and sediment-toxicity data were then used to develop site-specific concentration-response models and toxicity thresholds for PCBs, other COPCs, or various COPC mixtures using both empirically based sediment quality guidelines (SQGs; e.g., probable effect concentrations) and mechanistically based SQGs (equilibrium partitioning). The reliability and predictive ability of site-specific toxicity thresholds for PCBs and other COPCs were then evaluated to identify the most reliable basis for assessing risks to sediment-dwelling organisms at the site.

**MP241 Anniston PCB Site: 3. Inter-laboratory Comparison of SEM-AVS and Pore Water Metals Sampled by Peepers During Chronic Sediment Toxicity Testing** W. Brumbaugh, US Geological Survey, Columbia Environmental Research Center; N.E. Kemble, US Geological Survey, Columbia Environmental Research Center, USGS – Biological Resources Division, Columbia Environmental Research Center; J.L. Kunz, USGS; C.G. Ingersoll, USGS, Columbia Environmental Research Center; J.K. Stanley, J. Farrar, J. Steevens, US Army Engineer Research and Development Center; D. MacDonald, MacDonald Environmental Sciences Ltd.; S. Toms, US Environmental Protection Agency; M.S. Greenberg, USEPA Environmental Response Team. As part of a multi-party investigation, 32 sediment samples from a contaminated site near Anniston, AL, USA, were evaluated to assess bioavailability and toxicity of PCBs and other chemicals. Splits of sediments were tested concurrently by both US Army Corp of Engineers (USACE) and US Geological Survey (USGS) laboratories, in each of two toxicity testing cycles. As one component of the investigation, we evaluated repeatability of a mini-peeper method (in situ) for pore water sampling, and of sediment SEM and AVS concentrations in samples from exposure chambers tested by each laboratory. A 2.9-ml peeper was inserted into each sediment on test day 14, then removed on day 21. Afterwards, sediment from each of the same test beakers was transferred to a 60-ml glass jar, sealed, and stored at 4 °C for analysis for SEM-AVS. The SEM-AVS values were in good agreement for paired samples obtained from toxicity tests conducted by each laboratory for both testing cycles. Peeper metal concentrations in paired samples obtained from the first test cycle also were in close agreement; however, results from the second testing cycle were more variable. As evidenced by procedural blanks, the primary cause for variability in peeper samples from the second test cycle was trace-level contamination introduced during the transfer of peeper contents. Overall, this study demonstrated that consistent measurements for SEM, AVS, and pore-water metals are attainable in sediments tested and sampled by independent laboratories. Because of potential chemical changes that can occur during chronic whole-sediment toxicity tests, we recommend this type of pore water sampling approach. However, the transfer of peeper liquid contents must be performed using ultra-pure reagents and ultra-trace protocols to ensure reliable results.

**MP242 Evaluating the Read-Across Approach on CdTe Toxicity for CdTe Photovoltaics** S.W. Kaczmar, SUNY Upstate Medical Univ, Public Health and Preventive Medicine. Cadmium telluride (CdTe) is a semiconductor compound used in CdTe photovoltaic (PV) cells, an important thin film PV technology that is expected to reach nearly 4 GW in annual production capacity by 2014. Because there is limited energy and water use during operation, PV poses few environmental impacts and risks compared with other energy generation methods. The principal environment, health, and safety (EHS) issue for CdTe PV is the potential introduction of cadmium (Cd) compounds into the environment. This has been evaluated on a life cycle basis (Fthenakis, 2004) with regard to raw material, manufacturing, use, and decommissioning stages and found to produce minimal environmental emissions (0.02 g Cd per GWh). In evaluating risk from these emissions, toxicological data is often read-across from the parent element (Cd) to the compound (CdTe), because of limited specific toxicological

data on CdTe. However, recent toxicity studies indicate that this may not be appropriate. Zayed and Philippe (2009) evaluated acute inhalation and oral toxicities of CdTe in rats and found the median lethal concentration and dose to be orders of magnitude higher than that of Cd. Prior testing by Harris et al. (1994) showed no detectable effects of CdTe on male or female rat reproduction. Additional research on toxicity and ecotoxicity of CdTe is presented here, based on OECD, ASTM, and USEPA EHS test guidelines. CdTe was tested with bacterial reverse mutation assay (Ames test), and no mutagenic activity was found. This compares to positive mutagenicity results for Cd (Oberly et al., 1982). The bioavailability of CdTe was evaluated with a simulated gastric fluid (pH of 1.5) and yielded 11 mg of cadmium per g of cadmium telluride (~1%). This compares to a read-across value of 100% for cadmium chloride. Transformation and dissolution testing to evaluate aquatic toxicity yielded a concentration of Cd below the LOAEL of 18 ug/L. Acute toxicity was evaluated for Zebrafish over an exposure period of 96 hours at the limit of solubility for CdTe, and there was no toxic effect at aquatic saturation on fish. Overall, CdTe is differentiated from Cd, indicating that simple read-across from CdTe to Cd is not appropriate. Therefore, in addition to minimal life cycle environmental emissions, environmental risks from CdTe PV are minimized by differences in toxicity between CdTe and Cd.



**TP001 Bisphenol A in Drinking Water and Its Source Waters for North America and Europe** C.A. Staples, Assessment Technologies, Inc.; K.E. Clark, BEC Technologies, Inc.; G.M. Klecka, The Dow Chemical Company, Toxicology and Environmental Research Dept; S.S. Dimond, Sabic Innovative Plastics, Staff Toxicologist; N. Caspers, Currenta; S. Hentges, American Chemistry Council. A review of over 75 studies describing measurements of bisphenol A (BPA; also 4,4'-isopropylidene diphenol, CAS Registry No. 80-05-7) in finished drinking water and its source waters was conducted to characterize the concentration of BPA. BPA has been detected in a small fraction of drinking water and source water samples. The fraction of drinking water measurements reported as less than the detection limit is 95%, 54%, and 41%, for North America, Europe, and Asia, respectively. The highest measured concentrations in drinking water in North America, Europe, and Asia are: 0.099, 0.014, and 0.317  $\mu\text{g/L}$ . The median and 95<sup>th</sup> percentile concentrations of BPA in Asian drinking water are 0.020 and 0.16  $\mu\text{g/L}$ , while the median and 95<sup>th</sup> percentile concentrations in North America and Europe are limited by detection limits which range from 0.0002 to 5.1  $\mu\text{g/L}$ . In surface water/source water, the median and 95<sup>th</sup> percentile detection limits are: < 1 and < 1  $\mu\text{g/L}$  for North America, < 0.006 and < 5.1  $\mu\text{g/L}$  for Europe, and the median concentration for Asia is < 0.0155  $\mu\text{g/L}$ . In groundwater/source water the median and 95<sup>th</sup> percentile detection limits are < 1 and < 1  $\mu\text{g/L}$  for North America, while for Europe the median detection limit is < 0.001 mg/L. The 90<sup>th</sup> percentile concentration is < 0.073  $\mu\text{g/L}$  for Europe in groundwater/source water and is only an estimate since individual data points were not available for all studies. Oral reference doses (RfDs) for human exposure to BPA have been derived by several agencies, including USEPA, European Food Safety Authority, and Health Canada, and range from 16 to 50  $\mu\text{g/kg-body weight (bw)/day}$ . The lowest RfD, 16  $\mu\text{g/kg-bw/d}$  derived by Willhite et al. (2008), is based on no-observed adverse effect levels (NOAEL) of 5 mg/kg-bw/d identified for systemic toxicity in rats and mice and application of an uncertainty factor of 300. The USEPA provides a mean estimate of ingestion of drinking water of 0.61 L/d for children aged 1 to < 2 years and a body weight of 11.4 kg. Using the median and 95<sup>th</sup> percentile concentrations of BPA in drinking water in Asia of 0.020 and 0.16  $\mu\text{g/L}$ , respectively, and assuming 100% absorption, the estimated median and 95<sup>th</sup> percentile intakes of BPA due to ingestion of drinking water for children aged 1 to < 2 years are 0.0010 and 0.0086  $\mu\text{g/kg-bw/d}$ . These intakes represent less than 0.06% of the lowest RfD and relative margins of safety over the NOAEL of 580,000 or more.

**TP002 Chemical and Biological Analysis of Multi-residue Endocrine Disruptors in Wastewater Treatment Plants: A Perspective of Drinking Water Pollution** W. Lee, Univ of Texas-El Paso; R. De La Torre-Roche, A.C. Ortiz, T. Carrick, Univ of Texas at El Paso. Recent decades have brought an increasing concern of potential adverse human and ecological health effects resulting from emerging environmental endocrine disruptor compounds (EDCs), such as estrogens (17 $\beta$ -estradiol, estrone, 17 $\alpha$ -ethynylestradiol), bisphenol A (BPA), nonylphenol ethoxylates (NPEOS), and nonylphenol (NP). These chemicals are released directly to the environment after passing through wastewater treatment plants (WWTPs), which often are not designed to remove them in the effluent. The occurrence of the aforementioned compounds in surface water supplies is becoming of increasing concern worldwide, and has led to a growing awareness that animals, and perhaps human health and function in ecosystems might become negatively impacted by continued release of these compounds into the environment at low levels (ng L<sup>-1</sup>). To determine the concentrations EDCs and the possible impact of WWTPs discharge, chemical and biological analyses were used. EDCs concentrations in wastewater were analyzed by Stir Bar Sorptive Extraction-Thermal Desorption-Mass Spectrometry. Simultaneously, the estrogenic activity was quantified by a chemiluminescent yeast assay which was developed to test water directly without concentration. EDCs concentration and estrogenic activity in the influent were lower in WWTPs in El Paso compared to the plants located in Mexico. Concentrations in effluent were 200% to 8000% higher in Mexico for the majority of the EDCs in study compared to the plants in El Paso. NP and NPEOS were the compounds with the higher concentration ranging from non-detected to 8,144 ng L<sup>-1</sup> in influent. BPA levels in effluent water were below 581.6 ng L<sup>-1</sup> and for estrogen the levels were below 65.2 ng L<sup>-1</sup>. The removals of EDCs in WWTPs in El Paso were higher than 60% for the majority of the plants, and in Mexico lower than 60%. Estrogenic activity was removed 31% to 98% in WWTPs in El Paso. Insignificant removal of estrogenic activity was determined in plants from Mexico ranging from no removal

to 86%. Based on our observation, the WWTPs with at least secondary treatment process were able to remove EDCs more effectively for the EDCs analyzed in this study.

**TP003 Tracking Organics in the Santa Cruz River: Sources and Attenuation** A. Kahl, Univ of Arizona, Dept of Chemical and Environmental Engineering; L. Abrell, Univ of Arizona, Arizona Laboratory for Emerging Contaminants, Univ of Arizona, Soil, Water and Environmental Science; B. Arnold, Univ of Arizona; S. Snyder, Univ of Arizona, Chemical and Environmental Engineering; D. Jones, Univ of Arizona. It is now widely appreciated that trace organic compounds can affect the finished quality of water that is served to the public for potable use. Chemical fate during infiltration and underground transport to points of recovery is therefore relevant to the quality of delivered water. As reclaimed water begins to play a more important role in our regional water resource portfolio, interventions including natural processes during infiltration and underground transport will assume additional importance. Here we will attempt to (i) measure the levels of trace organic contaminants in several locations along the Santa Cruz River that serve as drinking water sources for major metropolitan areas (including Tucson), and (ii) measure the degree to which infiltration and underground storage/transport processes contribute to overall attenuation.

**TP004 USEPA/USGS Study of CECs in Source Water and Treated Drinking Water: Introduction and Quality Assurance/Quality Control** S.T. Glassmeyer, USEPA, Office of Research and Development, USEPA, NERL/MCEARD/CERB; E.T. Furlong, US Geological Survey, National Water Quality Laboratory; D. Kolpin, US Geological Survey; A.L. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; B. Benson, USEPA, Region 8; S. Boone, USEPA, Office of Chemical Safety and Pollution Prevention; O. Conerly, USEPA, Office of Research and Development; M. Donohue, USEPA; H. Mash, USEPA, Office of Research and Development; S. Pfaller, K. Schenck, J. Simmons, E. Varughese, S. Vesper, E. Villegas, M. Ware, USEPA, Office of Research and Development; V.S. Wilson, USEPA, ORD, NHEERL, TAD, Reproductive Toxicology Branch, USEPA, Office of Research and Development. Most studies of contaminants of emerging concern (CECs) are focused on a rather limited list of analytes (typically fewer than 20). While occurrence data in general is needed, information on the co-occurrence of compounds is particularly important to better assess the overall human and ecosystem exposure to these micro-pollutants. In 2010, the US Environmental Protection Agency (USEPA) and the US Geological Survey (USGS) began a joint study to measure more than 230 chemical and microbial CECs in the source water and treated drinking water of 20 drinking water treatment plants throughout the United States. This presentation serves as the introduction to a series of follow-up platform and poster presentations. In addition to the background information, this presentation will discuss the project's extensive quality assurance/ quality control samples including duplicate samples, laboratory fortified matrix samples (spikes) as well as inter-method comparisons.

**TP005 USEPA/USGS Study of CECs in Source Water and Treated Drinking Water: Perfluorinated Compounds** S. Boone, USEPA, Office of Chemical Safety and Pollution Prevention; T. Boone, C. Byrne, USEPA/OCSP/OPP/BEAD/Environmental Chemistry Branch; B. Guan, Univ of New Orleans, Dept of Chemistry; C. Vigo, J. Ferrario, USEPA/OCSP/OPP/BEAD/Environmental Chemistry Branch. Contaminates of emerging concern (CECs) are being found at trace levels (ppt) in domestic drinking water, and there is an increasing concern for the effects of their exposures to the United States population. The USEPA and USGS are performing a study of approximately 25 drinking water treatment facilities in the US for the detection of over 200 emerging chemical and microbiological contaminants. The source water for these facilities includes ground wells, reservoirs, and rivers. The contaminants of interest include: prescription and nonprescription pharmaceuticals and their metabolites, perfluorinated compounds, industrial chemicals, fragrances, polycyclic aromatic hydrocarbons, hormones, pesticides, detergent related chemicals, plant and animal sterols, phosphorus based flame retardants, nanomaterial, bacteria, fungi, protozoa, and viruses. The USEPA Environmental Chemistry Laboratory was tasked with the development of a methodology to detect seventeen perfluorinated compounds and the determination of their lowest concentration minimum reporting levels (LCMRLs). Using two extraction techniques (one for the source water/one for the drinking water), the LCMRLs (ng/L) for source/drinking waters for each compound were calculated from a low

of 0.035/0.052 ng/L for PFHxA to a high of 0.600/0.640 for PFOA for the carboxylic acids, and from a low of 0.034/0.033 ng/L for PFHxS to a high of 0.140/0.130 for PFOS for the sulfonates. Twelve water treatment facilities have been sampled to date, and of the twelve, all water samples had detectible levels from at least one PFC, and, in some, as many as 12 PFCs (9 carboxylic acids and 3 sulfonates). Of the 17 PFCs tested, the most prevalent PFCs measured were (in decreasing order): PFHxA, PFHpA, PFBS, PFBA, PFPeA, PFHxS, PFNA, PFOS, PFOA, PFDA, PFUnDA, and PFDoDA. The highest level of any PFC detected was PFBA (47 ng/L). The highest detection of PFOA and PFOS was 30 ng/L and 6 ng/L, respectively. In all but one case, the drinking waters had comparable levels to their source waters. For 5 source waters and 6 drinking waters (of the 12 facilities), the detectible levels of PFCs were all below 3 ng/L. The USEPA provisional health advisory for PFOA is 0.4 ppb (400 ng/L) and PFOS is 0.2 ppb (200 ng/L), and New Jersey has a health advisory for PFOA at 0.04 ppb (40 ng/L). None of the drinking water samples had detectible levels above any provisional health advisory in place at the current time.

**TP006 USEPA/USGS Study of CECs in Source Water and Treated Drinking Water: UPLC-IT/Orb Detection of Selected Endocrine Disrupting and Analogous Compounds** H. Mash, USEPA, Office of Research and Development; K. Schenck, US Environmental Protection Agency, ORD/NRMRL/WSWRD/WQMB; L. Rosenblum, Shaw Environmental & Infrastructure, Science and Technology Division. Numerous organic contaminants, including pharmaceuticals, endocrine disrupting compounds, personal care products, etc. have been detected in both surface and ground waters. Release into the environment is primarily due to their introduction from anthropogenic discharges and wet-weather runoff. Their presence in both source and finished drinking waters has led to concerns over the potential human health risks that may be associated with some of these organic contaminants, such as reproductive impairment, increased incidences of cancer and development of anti-biotic-resistant bacteria. This research investigates the effectiveness of a variety of drinking water treatments employed by full-scale treatment facilities to remove/alter selected contaminants. Solid-phase extraction followed by ultra-high pressure liquid chromatography/Ion Trap-Orbitrap mass spectrometry (SPE-UPLC-IT/Orb) was used to identify a group of organic contaminants, which included endocrine disrupting compounds (e.g., E1, E2, EE2, and E3), a pesticide (atrazine) and personal care products (e.g., triclosan). Results indicate atrazine is nearly ubiquitous in source waters, and persistent through most of the drinking water treatments investigated. In general, the other compounds investigated have either not been detected in the source water, or if present, have not been detected post-treatment.

**TP007 USEPA/USGS Study of CECs in Source Water and Treated Drinking Water: Assessment of Estrogenic Activity Using an In Vitro Bioassay, T47D-KBluc** V.S. Wilson, USEPA, ORD, NHEERL, TAD, Reproductive Toxicology Branch, USEPA, Office of Research and Development; N. Wrench, USEPA, ORD, NHEERL, TAD; K. Schenck, USEPA, ORD, NRMRL; H. Mash, USEPA, Office of Research and Development; L. Rosenblum, USEPA, ORD, NRMRL; S.T. Glassmeyer, USEPA, Office of Research and Development, USEPA, NERL/MCEARD/CERB. Scientists from the US Environmental Protection Agency (USEPA) and US Geological Survey (USGS) are collaborating on a research study to determine the presence of contaminants of emerging concern (CECs) in source and treated drinking waters collected from up to 30 drinking water treatment plants from across the United States. One study goal is developing tools that can be used with source waters and drinking water systems to evaluate the cumulative biological activity on critical endpoints. Our lab has developed an in vitro transcriptional activation assay, T47D-KBluc assay, which has been adapted for screening of environmental samples for estrogenic activity. These cells endogenously express both human ER alpha and beta and are stably integrated with an estrogen responsive promoter-luciferase reporter construct. This assay was confirmed by comparison to analytical chemistry to accurately predict estrogenic activity in water and effluent samples in a multi-lab collaborative study with the Global Water Research Coalition (GWRC). These assays have been successfully used to screen many types of samples including effluents from waste water treatment and cattle, dairy, swine and poultry operations. Further, with complex mixtures, it is important to understand how compounds with similar or different mechanisms of action would affect assay results. Several defined mixture studies have, therefore, also been conducted. These successes support the use of bioassays to evaluate

estrogenic activity in the proposed study. The focus of this part of the study is detection of potential estrogen-mediated activity in extracts from source and treated drinking water. Water from plants using various treatment processes will be tested. In vitro results from the current study will be compared to chemical identification of endocrine active compounds. Initial testing of control and mock-up sample extracts have confirmed appropriate responses and provide a high degree of confidence that the application of the in vitro assay will be possible. The current successes in evaluating endocrine activity via the estrogen pathway indicate that bioassays targeting other pathways could be developed and used to evaluate water samples as well. Disclaimer: This abstract does not necessarily reflect USEPA policy

**TP008 Life-Cycle Exposure of *Daphnia magna* to Environmentally Relative Mixtures of Pharmaceuticals** D.N. Wolfe, S. Richards, Univ of Tennessee at Chattanooga; M. Hanson, Univ of Manitoba. Due to the global detection of pharmaceuticals in surface water, risk assessments require toxicity testing to be performed to close knowledge gaps for effects assessment regarding impacts on aquatic food web dynamics. The widespread use of pharmaceuticals has resulted in mixture concentrations of mg/l in effluent and ug/L concentrations in surface water. Their potential toxicological effect on fresh water ecosystems remains largely unknown, especially as complex mixtures. Toxicity data on the effect of pharmaceuticals has expanded in the past decade, but has mainly focused on single, acute pharmaceutical exposures, not environmentally realistic mixtures. By determining the threshold of response for environmentally relevant mixtures, the risk that these compounds may pose to the environment will be determined more accurately. In 2008, thirteen pharmaceuticals were quantified in the Tennessee River, USA and its tributaries, ranging from 0.1757 to 0.0028 ug/l. Using the same ratios of individual compounds, but increased concentrations, the present study conducted chronic life cycle toxicity test on the cladoceran *Daphnia magna*. Tests were performed using 10x, 100x and 1000x the concentrations detected in the Tennessee River, resulting in a total concentration of 6.031, 60.31, and 603.1 ug/l of total pharmaceutical exposure, respectfully. Mortality, time to first brood, size and fecundity were used as endpoints of toxicity. Test solutions were renewed three times a week and neonate removed each day until the conclusion of the experiment. *D. magna* showed a statistically significant decrease in the number of neonate produced when exposed to 100x and 1000x ( $p < 0.003$ ). When *D. magna* was exposed to the 10x concentration, no significant decrease in neonate production was observed. Neither time to first brood nor size was affected at 10x, 100x, or 1000x. Studies are on going to pinpoint the threshold of effects occurring between 10x and 100x. These data will provide risk assessors more accurate data when analyzing non-lethal effects of pharmaceutical mixtures on aquatic ecosystems.

**TP009 Monitoring of Perchlorate in Diverse Foods and Its Estimated Dietary Exposure for Korea Populations** J. Lee, Pusan National Univ, Dept of Civil and Environmental Engineering; S. Oh, Pusan National Univ; W. Sim, Pusan National Univ, Korea Testing & Research Institute; J. Oh, Pusan National Univ, Environmental Engineering. Perchlorate is considered as a new emerging pollutant because of its health concern and ubiquitously detection. Perchlorate has been found in food and the main exposure pathway of perchlorate is the ingestion. Therefore, determination of perchlorate in food is particularly important. In 2004, USFDA conducted a large survey to monitor the perchlorate levels in food samples. They analyzed 775 samples from 27 food groups suspected contamination. From 2005-2006, they analyzed perchlorate in American's favorite foods to estimate perchlorate dietary exposure amount for general populations. They reported that several foods were contaminated with perchlorate and the levels of perchlorate ranged from 0.15 to 92.4 ppb. However, there is no other large-scale study, globally. Therefore, we carried out a large-scale of monitoring on perchlorate level in Korean food to understand the level of perchlorate and to assess the exposure amount of perchlorate by ingestion for Korean people. Total 663 samples from 39 kind different food groups purchased from Korean market were analyzed and target sample groups were selected based on dietary habits of general and/or specific age population. The preparation of perchlorate in food samples was conducted by modified US FDA and EPA methods. The recoveries of perchlorate in food samples ranged from 77.1 to 118.3% (RSD 0.5-14.8%). Measurable amounts of perchlorate were detected in 411 samples. The mean concentration of the 6 types of food samples has the following trend (ppb): fruit & vegetables (7.92) > dairy (6.34) > processed product (3.52) > alcohol & beverage (1.91) > meat & egg (0.98) > fishes &



shellfishes (0.95). These levels of Korean food samples were similar or lower than other previous studies. The exposure dose of perchlorate was calculated with analyzed perchlorate levels in domestic food samples in this study. The calculated daily perchlorate dose to Korean adult was 0.04  $\mu\text{g}/\text{kg}\cdot\text{day}$  based on average concentration and 0.15  $\mu\text{g}/\text{kg}\cdot\text{day}$  based on maximum concentration, which was pretty low compared to RfD (0.7  $\mu\text{g}/\text{kg}\cdot\text{day}$ ) value suggested by US NAS. This result indicates that the current perchlorate exposure to Korean people by domestic food consumption is safe. However, the daily perchlorate dose for Korean infants (0.14  $\mu\text{g}/\text{kg}\cdot\text{day}$  based on average concentration) was higher than adults due to high levels of perchlorate in dairy products, indicating management of perchlorate exposure in infants group needs to be considered.

**TP010 Simultaneous Removal of Dimethyl Phthalate and Dissolved Organic Matter Using an Aminated Hypercrosslinked Resin** C. Shuang, Y. Xue, A. Li, Nanjing Univ. Water contaminations caused by endocrine disrupting chemicals (EDCs) and dissolved organic matters (DOMs) threat humans' health. An aminated hypercrosslinked resin was designed for simultaneous removal of both EDCs and DOMs. The obtained resin (NDA-8) was prepared and characterized. It was found that the hydrophobic hypercrosslinked resin turned to hydrophilic after amination with the declined in contact angle from 131.3° to 72.7°. The resin had a surface area of 580  $\text{m}^2/\text{g}$  and ion exchange capacity (IEC) of 2.35  $\text{mmol}/\text{g}$ . The performance of NDA-8 was investigated for the adsorption of dimethyl phthalate (DMP) and tannic acid (TA) from aqueous solutions, which are respectively used as model compound for EDCs and DOMs. Activated carbon (F400D), anion exchange resin (D730) were also used in adsorption for comparison. The adsorption capacities of DMP onto NDA-8 and F400D were 370, 256  $\text{mg}/\text{g}$  at 298 K, respectively, while the adsorption onto D730 could be negligible. The capacities of TA onto NDA-8, F400D and D730 were 331, 117, 526  $\text{mg}/\text{g}$  at 298K, respectively. The results indicated that the uptake of DMF was contributed on adsorption and TA mainly attributed to anion exchange. Due to the combination of adsorption and anion exchange for NDA-8, the binary adsorption performed the high removal efficiencies (>90%) of both DMF and TA at both initial concentrations of 10  $\text{mg}/\text{L}$  at 298K.

**TP011 Sorption of Micropollutant Estrone to Magnetic Hypercrosslinked Resins** M. Zhang, C. Shuang, Nanjing Univ, School of Environment; Q. Zhou, Nanjing Univ, School of Environment; A. Li, Nanjing Univ, School of Environment; J. Qiu, Shanghai Jiao Tong Univ, School of Life Sciences & Biotechnology. The hypercrosslinked magnetic polymer beads with different pore structures were obtained using suspension polymerization and hypercrosslinking reaction. The obtained resins could be easily and rapidly separated owing to their superparamagnetic characteristics. Moreover, they had abundant micropore and mesopore distribution with the large specific surface area of from 600 to 1200  $\text{m}^2/\text{g}$ . The investigation of estrone sorption on the resin showed that both the pore volume and the pore diameter were the main factors in the sorption and desorption of estrone removal.

**TP012 Preparation of a Novel Magnetic Absorbent for the Removal of Tetracycline in the Aquatic Environment** Q. Zhou, Nanjing Univ, School of Environment; J. Qiu, Shanghai Jiao Tong Univ, School of Life Sciences & Biotechnology; M. Zhang, Nanjing Univ; A. Li, Nanjing Univ, School of Environment. A novel magnetic absorbent was synthesized by membrane emulsification-suspension polymerization (MESP) method. The obtained magnetic polymer microspheres exhibited distinct superparamagnetic characteristics, resulting in easy and rapid separation properties in the applications. Moreover, the absorbent was well monodispersed with the low-micrometer diameter and ultra-large specific surface area (more than 1200  $\text{m}^2/\text{g}$ ). These advantages of the magnetic absorbent led to the excellent adsorption characteristic for organic micropollutant removal. It was found that the magnetic absorbent had not only similar adsorption capacity and dynamic property to the commercial powder activated carbon (PAC) 1240AC, but also much better desorption behavior than PAC. Furthermore, the competitive adsorption investigation of tetracycline and natural organic matter (NOM) showed that the existence of NOM had little effect on the removal of tetracycline using the magnetic absorbent. This could be explained by its controllable pore structure, uniform particle and low-micrometer size.

**TP013 The Influence of Life History on the Sublethal Effects of Sea Lamprey Parasitism on Lake Trout** S. Smith, Michigan State Univ, Fisheries and Wildlife; C.A. Murphy, Michigan State Univ, Dept of Fisheries and Wildlife, Lyman Briggs College, Michigan State Univ, Lyman Briggs College; F. Goetz, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences; S. Sitar, Michigan Dept of Natural Resources, Marquette Fisheries Research Station; R. Bergstedt, USGS Great Lakes Science Center, Hammond Bay Biological Station. Sea lamprey (*Petromyzon marinus*) invasion of the Great Lakes has been linked directly to lake trout (*Salvelinus namaycush*) population decline through the effect of parasitism mortality. Less well understood are the sublethal effects of sea lamprey parasitism on lake trout. This study aims to identify and quantify sublethal effects of parasitism, such as changes in hepatic gene expression, fecundity, and growth and to determine if life history can influence such effects. We examined the effects of parasitism on two different lake trout morphotypes: siscowet and lean. Siscowets are found in Lake Superior at depths greater than 100m and have higher weights, lipid levels, and sea lamprey wounding rates than leans. Leans are found in shallow water throughout the Great Lakes. Siscowet and lean lake trout (n=64) were subjected to sea lamprey parasitism for one to five days and analyzed for changes in blood hormones, pituitary gonadotropin mRNA, lipid levels, fecundity, and hepatic gene expression. Wild Lake Superior lake trout (n=58) were also collected and sampled similarly to determine the range of responses experienced by fish in the field. Preliminary results suggest that as the length of time of parasitism increases, the hepatosomatic index decreases linearly, with leans showing a more drastic reduction. Changes in hepatic gene expression in response to parasitism were also observed with significant differences between lean and siscowets. Future work plans to incorporate changes in life history specific sublethal effects (such as plasma sex steroids) into models to link to population-relevant endpoints such as changes in fecundity and growth. Current models that estimate spawning stock biomass per recruit do not take into account the likely decrease in reproductive ability due to sublethal sea lamprey wounding and may be underestimating the full effect of sea lamprey parasitism on specific lake trout populations.

**TP014 Effects of Munitions and Breakdown Products on Activation of Peroxisomal Proliferator Activated Receptors and Their Heterodimer Retinoid X Receptor** D.R. Johnson, K.B. Donohue, US Army Engineer Research and Development Center, Environmental Laboratory; C. Ang, Badger Testing Services; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division. Exposure to munitions compounds has been shown to alter energy stores and biomolecules in wildlife. For example, 2,6-dinitrotoluene (2,6-DNT) impacts the expression of genes that facilitate energy metabolism including both gluconeogenesis and lipid metabolism in northern bobwhite (*Colinus virginianus*). This appeared to occur by 2,6-DNT-mediated suppression of peroxisome proliferator activated receptor gamma (PPAR $\gamma$ )-regulated genes. PPAR is a class of nuclear receptors (i.e., transcription factors) that, when bound by endogenous and xenobiotic ligands, activate the transcription of regulated genes. While primarily found in several different tissues and activated by different ligands, PPARs are generally involved in changes of lipid and glucose homeostasis and inflammation. When bound by ligands, PPAR nuclear receptors heterodimerize with the retinoid x receptor alpha (RXR $\alpha$ ). The objective of this project was to determine the role of military munitions in PPAR-mediated gene activation. PPAR alpha and gamma activation by military munitions (0.1-10  $\text{mg}/\text{l}$ ) and their breakdown products after 24 h exposure were detected by cell-based nuclear receptor activation kits. Cells were also treated with positive controls (GW590735 for PPAR $\alpha$ , rosiglitazone for PPAR $\gamma$ , and 9-cis retinoic acid for RXR $\alpha$ ), all of which activated their respective receptor-reporter system by 10-70 fold. No munitions significantly activated PPAR $\alpha$ , but 2,4,6-TNT and respective breakdown products (e.g., 2-amino-4,4-dinitrotoluene, 4-amino-2,6-dinitrotoluene) downregulated PPAR $\alpha$  by 40-60%. For PPAR $\gamma$ , 2,4,6-trinitrotoluene (TNT) strongly activated the receptor in a dose dependent manner, with 6.8-fold induction by 10  $\text{mg}$  TNT/l. The munition 2,4-DNT was a weak PPAR $\gamma$  activator (65% increase) at 10  $\text{mg}/\text{l}$ . In contrast, 2,6-DNT and 4-amino-2,6-DNT decreased PPAR $\gamma$  activation by 50-60%. For RXR $\alpha$ , TNT and respective breakdown products at 10  $\text{mg}/\text{l}$  decreased RXR $\alpha$  activation by 40-70%. Triazole munitions (e.g., RDX) and new insensitive munitions (e.g., dinitroanisole) did not activate either PPAR receptor. These data demonstrate the ability of munitions to regulate nuclear receptor activation, resulting in



the differential regulation of numerous genes involved in lipid and glucose homeostasis. Trends in PPAR activation can be seen based on commonalities in chemical structures. These data may provide insight into molecular initiation events that can be linked to adverse outcomes at increasing levels of biological organization.

**TP015 Relationships of Fluorescence, Dissolved Oxygen, and Analytical Chemistry Measurements as Indicators of MC252 Oil in the Gulf of Mexico Water Column** M. Johns, Exponet, Inc.; M.R. Edwards, Exponet; R. Atlas, Univ of Louisville; J. Harney, T. Thompson, Cardno Entrix. The Deepwater Horizon accident (April 20, 2010) was unique in that it originated from a water depth of approximately 1,500 m. The oil release continued until July 15, 2010 when the well was capped. Physical and chemical dispersion of the MC252 oil resulted in a cloud of fine droplets at approximately 1,100–1,300 m that generally moved in a southwesterly direction while larger droplets moved to the surface and formed a slick that moved toward the shoreline. Numerous vessels were equipped with conductivity, temperature and depth (CTD), dissolved oxygen, fluorometry, and deep water collection capabilities to evaluate and track the subsea dispersed oil. Field fluorometry measurements were collected throughout the water column from early May to December 2010 at approximately 2,000 locations, including before and after the well was capped. These measurements were used to track the location of the subsea dispersed oil in real time. Water chemistry samples were collected and analyzed to quantify the field measurements. This poster presents an evaluation of the associations between the fluorometry, dissolved oxygen, and analytical water chemistry results over that period of time and in all directions and distances about the wellhead, including a summary of the quantity of data collected from this effort. The large quantity of data evaluated indicated fluorescence is not consistently associated with a depression in dissolved oxygen, and a sharp change in either fluorescence or dissolved oxygen is not always associated with the presence of polycyclic aromatic hydrocarbons (PAHs).

**TP016 Toward the Full Chemical Characterization of Dispersants Used for Oil Spills** M. Azzam, Univ of Houston-Clear Lake; C. Zhang, Univ of Houston-Clear Lake, Univ of Houston-Clear Lake, Environmental Science. Dispersants are employed in oil spill control under the general assumption that the active ingredient (surfactants) will reduce the interfacial tension and promote the break-up of the oil slick into fine droplets. Limited data are available to the public from the MSDS sheets on the chemical characterization of commercial dispersants, since the exact compositions are commonly considered to be a trade secret. However, such information is critical for the fate and transport modeling of dispersed crude oil, development of clean-up strategies, and analyses of affected environmental and biological media. Our work attempted to perform non-targeted trace analyses of metals, volatile and semi-volatile organic compounds that may be of importance for the chemical and toxicological evaluation of dispersant use in oil spill scenarios. Four commercially available dispersants were analyzed for inorganic and organic components using an array of instruments including ICP, GC-MS, LC-MS-ESI coupled with UV-DAD and fluorescence detectors (FLD), and LC-MS with accurate molecular mass time-of-flight (TOF) detector. GC-MS analysis of methanol prepared aliquot shows light aliphatic alcohols, ethers and ketones (e.g., 2-butoxy ethanol, 2- and 3- hexanol, 2- and 3- hexanone), whereas hexane extracts show a range of saturated, unsaturated aliphatics and cyclic hydrocarbons (e.g., 1,2,3-trimethyldiaziridine, 1-hexene, 2,5-dihydro-3-methylfuran, cyclohexane, undecane). LC-UV-FLD with an Acclaim surfactant column (4.6×150 mm, dp = 5 µm, Dionex) in an isocratic mode (60/40 acetonitrile, H<sub>2</sub>O in 0.1 M NH<sub>4</sub>AcO) results in a group of pure and co-eluting dispersant components. When NH<sub>4</sub>AcO was used as the modifier in both the negative and positive ionization modes, LC-MS reveals molecular ions of surfactant sorbitan laurate and ethoxylates. TOF further reveals molecular formulas for the major ions in the range of m/z 200 – 500 (e.g., m/z 289, 317, 345, 428, 472). Since many of these compounds are not described in the MSDS, the implications of our findings regarding the evaluation of contaminant fate, transport and toxicity to concerned groups (researchers, practitioners and policy makers) of Gulf oil spill will be discussed.

**TP017 Measurement of Oil Dispersant Ingredients Dioctyl Sodium Sulfosuccinate (DOSS) and Dipropylene Glycol Monobutyl Ether (DPnB) in Seawater and Sediment** M.J. Benotti, Battelle, Analytical and Environmental Chemistry; F. Pala, Battelle; J.R. Thorn, Battelle, Applied

Research and Laboratory Operations. Dispersants such as the Corexit have been applied to oil spills for many years. This talk discusses the development and application of two analytical methods pertinent to studying the fate and transport of Corexit dispersants. The first method is used for sensitively measuring dioctyl sodium sulfosuccinate (DOSS; CAS# 577-11-7) in seawater and sediment, the primary surfactant in Corexit. Aqueous extraction employs solid phase extraction, and the sediment extraction employs accelerated solvent extraction. Analysis is conducted using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with negative mode electrospray ionization. This method benefits from use of a deuterated surrogate standard (DOSS-d34) and a <sup>13</sup>C-labeled internal standard (DOSS-<sup>13</sup>C4). Thus, method variability due to loss during extraction and/or ionization suppression/enhancement is mitigated. Method detection limits are in the low ng/L range for seawater samples and the low ng/g range (dry) for sediment. The second method is used for sensitively measuring dipropylene glycol monobutyl ether (DPnB; CAS# 29911-28-2) from seawater and sediment, another ingredient in Corexit. Aqueous extraction employs liquid-liquid extraction, and the sediment extraction employs use of a shaker table. Analysis is conducted using gas chromatography coupled with mass spectrometry (GC-MS) operated in selected ion monitoring mode. Method detection limits are less than 10 ng/L for seawater samples and less than 1 ng/g (dry) for sediment. Both approaches are calibrated against laboratory experiments studying the fate of Corexit following application. The partitioning of Corexit between water, sediment and biota will be discussed. Ultimately the use of both the DOSS and DPnB methods affords a more complete understanding of the ultimate fate of Corexit in the coastal and marine environment as compared to approaches focusing on a single ingredient.

**TP018 Toward the Full Chemical Characterization of Dispersants Used for Oil Spills** M. Azzam, Univ of Houston-Clear Lake; C. Zhang, Univ of Houston-Clear Lake, Univ of Houston-Clear Lake, Environmental Science. Dispersants are employed in oil spill control under the general assumption that the active ingredient (surfactants) will reduce the interfacial tension and promote the break-up of the oil slick into fine droplets. Limited data are available to the public from the MSDS sheets on the chemical characterization of commercial dispersants, since the exact compositions are commonly considered to be a trade secret. However, such information is critical for the fate and transport modeling of dispersed crude oil, development of clean-up strategies, and analyses of affected environmental and biological media. Our work attempted to perform non-targeted trace analyses of metals, volatile and semi-volatile organic compounds that may be of importance for the chemical and toxicological evaluation of dispersant use in oil spill scenarios. Four commercially available dispersants were analyzed for inorganic and organic components using an array of instruments including ICP, GC-MS, LC-MS-ESI coupled with UV-DAD and fluorescence detectors (FLD), and LC-MS with accurate molecular mass time-of-flight (TOF) detector. GC-MS analysis of methanol prepared aliquot shows light aliphatic alcohols, ethers and ketones (e.g., 2-butoxy ethanol, 2- and 3- hexanol, 2- and 3- hexanone), whereas hexane extracts show a range of saturated, unsaturated aliphatics and cyclic hydrocarbons (e.g., 1,2,3-trimethyldiaziridine, 1-hexene, 2,5-dihydro-3-methylfuran, cyclohexane, undecane). LC-UV-FLD with an Acclaim surfactant column (4.6×150 mm, dp = 5 µm, Dionex) in an isocratic mode (60/40 acetonitrile, H<sub>2</sub>O in 0.1 M NH<sub>4</sub>AcO) results in a group of pure and co-eluting dispersant components. When NH<sub>4</sub>AcO was used as the modifier in both the negative and positive ionization modes, LC-MS reveals molecular ions of surfactant sorbitan laurate and ethoxylates. TOF further reveals molecular formulas for the major ions in the range of m/z 200 – 500 (e.g., m/z 289, 317, 345, 428, 472). Since many of these compounds are not described in the MSDS, the implications of our findings regarding the evaluation of contaminant fate, transport and toxicity to concerned groups (researchers, practitioners and policy makers) of Gulf oil spill will be discussed.

**TP019 Simulation of DWH Oil/Gas Releases Including Dispersant Application, with Focus on Droplet Size Distributions Experimental Concept and Initial Results** P.J. Brandvik, O. Johansen, F. Leirvik, P. Dalving, SINTEF, Materials and Chemistry, Marine Environmental technology. During the initial phase of the Deep Water Horizon (DWH) incident oil and gas were released from two locations on the broken riser and one on the drill pipe. Later the riser was removed and oil and gas were released from a single location on top of the BOP. Subsea dispersant was injected into

the released oil and gas stream at varying locations. The size and distribution of oil droplets is believed to affect such factors as the time required for oil to rise to the surface, the location where it surfaces, dissolution in the water column, and entrainment of the smallest droplets beneath deepwater density layers. There were no immediate deepwater in situ measurements of oil droplet sizes at the various release points, or at the location of subsurface dispersant injection into the turbulent deep water release. The first focus area of this project is to simulate the release of oil and gas from a hypothetical subsurface blowout in order to increase our knowledge regarding the droplet size and distribution of released oil. The second focus area is to study different application techniques for deep water dispersant application and their influence on droplet size and distribution. A Tower tank is established at SINTEF for this purpose. The tank is 6 meters high, 3 meters wide and holds 40m<sup>3</sup> of natural sea water. The tank is equipped with an advanced system for releasing oil and gas, and monitoring oil droplet size distributions and oil concentrations in the released plume. The droplet size distributions are measured with three independent in-situ methods (laser particle sizer, particle visual microscope and a macro camera with a blue-laser focusing plane). Different methods are studied to inject dispersants into the hydrocarbon stream. Although the tower cannot reproduce the temperature and pressure conditions encountered in a deep water release, the experimental conditions will exceed bench scale tests by at least one order of magnitude. We will review the assumptions needed to mathematically scale these results to the conditions involved in a deepwater release. Our aim is to use the broad range of experimental conditions possible with this basin to provide more confident extrapolations to full scale conditions. The experimental concept of the tower basin and initial results with oil alone, oil/gas and with dispersant will be presented.

**TP020 Distribution and Fate of PAH and Chemical Dispersants in the Water Column Following the Deepwater Horizon Oil Spill** P. Boehm,

Exponent, Environmental & EcoScience Group, Exponent, Environmental Group, Exponent, Inc., Environmental & EcoScience sGroup; L. Cook, Exponent, 1 Clock Tower Place; L. Royer, Exponent, Inc.; R. Barrick, Infinity Solutions. An assessment of the chemical distributions of key chemicals related to the MC252 oil spill in approximately 10,000 offshore water samples (>3 miles from shore) that comprise a 4-dimensional (area x depth x time) data set from several key water column zones are discussed in this presentation. These samples were taken during the release period (April 20 through July 15, 2010) and in the post-release period (July 15 through the end of December, 2010). Total polycyclic aromatic hydrocarbon (TPAH) concentrations and distributions of the chemical dispersant, Corexit 9500, using two chemical tracers (DPnB and DOSS) are discussed. TPAH were found with a geometric mean of less than 0.01 ppb concentrations ranging from not detected (ND) to 146 mg/L (parts per million), the latter sample collected directly from the riser plume at 1524m water depth. Eighty-five (85) percent of all samples were at Total PAH (TPAH) concentrations of < 0.1 ppb, essentially at or near background levels. The highest concentrations of PAH (i.e., >1ppb) were largely observed only during the release (May-July) and the higher concentrations close to wellhead. The highest concentrations observed in the surface waters (0-10 m), not at depth, likely due to re-entrainment from the surface. PAHs in subsurface water masses including the subsurface layer from 1100 to 1300 are rapidly attenuated; post-release concentrations quickly return to low (background) levels. Mean concentrations (exposures) were uniformly low despite rare and sporadic high concentrations. Within the 1100-1300 m depth range TPAH seldom exceeded 10ppb with the highest concentration of 23 ppb TPAH and a geometric mean value < 0.1 ppb. Deepwater concentrations of TPAH attenuated rapidly with distance from the release point (the wellhead) and were seen to reach < 1.0 ppb within 15-20 miles in all directions other than to the southwest, where a small number of samples exceeded 1ppb out to 40 miles. The detailed compositions of 2 through 5 ringed parent and alkylated PAHs of TPAHs varied widely; the range of these compositions and the association with dissolved and droplet fractions will be discussed. Chemical dispersant concentration (DPnB) distributions generally mimic those of the TPAH in the water samples. The range during the release was from ND to < 1000 ppb with high concentrations rarely exceeding 10 ppb. DOSS concentrations during the release were similar to DPnB, but after the release the DOSS concentrations appeared to remain higher (< 10 ppb) for longer and over a wider range than did the DPnB. Explanations for this apparent decoupling of DPnB and DOSS tracers of the dispersant will be discussed.

**TP021 Chemical Composition of Different Weathering Stages of the Macondo Oil, and Their Water Accommodated Fractions** L. Faksness, SINTEF, Marine Environmental Technology; P.S. Daling, SINTEF, Marine Environment Technology; K. Almaas, SINTEF, Marine Environmental Technology. At the SETAC meeting in Pensacola in April 2011 initial results on the chemistry on laboratory weathered Macondo oil samples were shown. This presentation gives the detailed results on each of the laboratory generated weathered oil residues, which included quantification of more than 200 compounds (including volatiles, PAHs, saturates, and biomarkers). Furthermore, an inter-laboratory comparison between three laboratories is documented. The chemical composition of the water accommodated fractions (WAFs) from selected numbers of these weathered samples will be reported, as the chemical profile of the WAF is very unlike that of the parent oil due to different water solubilities of the various compounds. The Macondo oil is a light paraffinic crude oil, where e.g., 50-55 wt% will evaporate within 5 days on the sea surface. Preparation of WAF has been performed under controlled conditions following the guidelines established by the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF). The CROSERF testing protocols were developed to standardize WAF preparation, laboratory exposures to aquatic organisms, and analytical chemistry measurements used to determine the acute toxicity of the water soluble components in the oil. The WAF is of special interest because such components dissolved from an oil slick or from the dispersed oil droplets beneath a slick are known to have a high bioavailability to marine organisms and therefore have a potential for causing acute toxic effects. Different weathering degrees have been used in preparing the WAFs to illustrate "snapshots" in the dynamic process of weathering and dissolution occurring during a spill situation: Fresh and artificially weathered oils from 150 °C+ (compounds with boiling point below 150 °C is topped off) to 275 °C+, where the 150°C+ residue is assumed to be similar to the surfacing oil, and the 275°C+ residue to approximately 3 to 5 days on sea. An oil-to-water ratio of 1 to 10000 (100 mg oil/L water), which is considered to be a realistic approach in an oil spill release after treatment of chemical dispersants, are used.

**TP023 Applications of Using Hyperspectral Data for Oil Spill Monitoring and Assessment** D. Faust, Texas Tech Univ/TIEHH, Dept of Environmental Toxicology; L. Cong, Texas Tech Univ, Dept of Electrical Engineering; P.N. Smith, Texas Tech Univ/ TIEHH, Environmental Toxicology; S. Cox, Texas Tech Univ, Dept of Environmental Toxicology; B. Nutter, Texas Tech Univ, Dept of Electrical Engineering; D. Liang, Texas Tech Univ.

Monitoring and assessment of oil spills has traditionally relied on visual observations made either in the field or via remotely sensed imagery. Recent advances in sensing technologies and computational capabilities offer new opportunities for developing reliable, quick and automated detection and mapping methods to better support response, recovery planning, and impact analysis. Following unintended releases of oil, degradation processes quickly and dramatically change the chemical composition of crude oil. Thus, its physical form, toxicity, and spectral image signature will also evolve. We hypothesized that spectral signatures of oils were unique and, in response to weathering, would change over time in a manner that would allow hyperspectral imaging to be used as an oil spill monitoring and assessment tool. Using a FieldSpec3 Max Spectrometer Field Spectroscopy Environmental Analysis system, we measured solar reflectance from fresh West Texas sour crude and weathered crude oil collected from the Gulf of Mexico at approximate thicknesses of 0, 0.5, 1, 2, 4, and 8 mm on the surface of sea water in a microcosm environment. Crude oils were exposed to environmental conditions, and hyperspectral solar reflectance was measured weekly for ten weeks. Solar reflectance was different among the various thicknesses and sources of oil. For the sour crude oil at thicknesses of 1, 2, 4, and 8 mm, the reflectance spectrum was relatively uniform throughout the complete wavelength range, which was quite different from the spectrum of seawater. Generally, there was less reflectance with increasing surface oil thickness. Spectrally, the 0.5 mm oil surface exhibited spectral features from both thick oil surfaces and seawater and thus can be considered a linear mixture of both thick oil surfaces and pure seawater. The hyperspectral signatures of the oils changed with time, likely due to weathering and degradation processes. Generally we found that the spectrum of oil surfaces became very similar to that of seawater following degradation processes. Thick surfaces were also observed to degrade slower than thin surfaces. Correlation of in-situ data collected in this study with hyperspectral aerial or satellite imagery has the potential to yield a powerful tool for long-term monitoring, assessment, and management of future oil spills.

**TP025 PAHs from MC-252 in Whole Blood and RBCs from Live-captured Birds Using Ultra Performance Liquid Chromatography (UPLC): Method Development/Assessment** C.R. Perkins, Univ of Connecticut, Center for Environmental Sciences and Engineering; A. Provatas, Univ of Connecticut, Center for Environmental Sciences and Engineering; W. Seegar, M. Yates, G. Doney, Earthspan; D. Evers, Biodiversity Research Institute. The explosion of the Deepwater Horizon oil platform on April 20, 2010 resulted in an unprecedented release of crude oil in the Gulf of Mexico. Much of the oil and the dispersants used impacted salt marshes and beaches around the Gulf, with numerous species of birds documented as being exposed. While the use of dispersants in an oil spill response involves tradeoffs between effects to the shoreline and effects to pelagic and deep-sea environments, relatively little is known on the effects that the oil-associated polycyclic aromatic hydrocarbons (PAHs) have upon the health of avian populations. Circulating blood-borne, parent PAHs can provide a direct link for exposure assessment and reconstruction since they are not as affected by differences in metabolism and excretion. There are a couple of challenges in analyzing whole blood and red blood cells (RBCs) from live-captured birds that make quantitation more difficult; minimal sample volume (0.1- 0.5 ml) can be obtained without being detrimental and the target analytes are less abundant by volume in circulating RBCs in contrast to whole blood. We developed a novel method for the analysis of 16 PAHs utilizing ultra-performance liquid chromatography coupled to photodiode array, fluorescence, and tandem mass spectrometry detectors. This rigorous method obtained good recoveries of standard reference material (60-95%), matrix spikes (60-95%), calibration verifications (90-95%), and surrogates (85%) while obtaining good sensitivity of at least 5ng/g for PAHs. This method was developed in support of an ongoing study examining uptake and exposure in migrating peregrine falcons and other birds. Funding for a portion of this study was provided by Peter Jenny and The Peregrine Fund.

**TP026 Assessment of Water, Sediment, and Oyster PAH Concentrations and RNA and Protein Expression Following the Deepwater Horizon Oil Spill** M. Dailey, J. Corrales, C. Thornton, Univ of Mississippi, Environmental Toxicology; H. Patterson, Univ of South Alabama, Marine Science; A. Boettcher, Univ of South Alabama, Biology; R. Carmichael, Dauphin Island Sea Lab; M. Slattery, Univ of Mississippi, Pharmacognosy and Environmental Toxicology; K.L. Willett, Univ of Mississippi, Environmental Toxicology Research Program, Univ of Mississippi, Dept of Environmental Toxicol. On April 20, 2010, BP's Deepwater Horizon oil rig exploded leaking over 200 million gallons of crude oil into the Gulf of Mexico for 100 days. Exposure to oil-associated polycyclic aromatic hydrocarbons (PAHs) in the water and sediment could severely impact the aquatic organisms inhabiting the Gulf of Mexico (i.e., developmental defects, reproductive effects, death, etc.). Therefore, water, sediment and oyster, *Crassostrea virginica*, samples were collected approximately bimonthly between May 26 and November 30, 2010 from three sites along the Alabama Gulf Coast, namely, two sites in Mobile Bay (Denton and Sand at 1 or 0.1 m above the bay floor) and one near Perdido Bay. Water, sediment, and oysters were extracted for quantification of 24 PAHs by GC/MS. A possible correlation between PAH exposure and biological processes was investigated by studying RNA and protein expression in oyster gill by qRT-PCR and bottom-up shotgun proteomics, respectively. In bottom-up proteomics, proteins were digested into peptides and analyzed by LC/MS. Expression of stress indicators (e.g., catalase, superoxide dismutase, 14-3-3 proteins, heat shock proteins, etc.) at the transcriptomic and/or proteomic levels were measured. The concentration range for total PAHs in water was 3.46-1240 ng/L. The highest water total PAH concentrations were observed on 6/28/10 for Sand (1 m), 8/4/10 for Sand (0.1 m), 7/21/10 for Denton (1 and 0.1 m), and 9/9/10 for Perdido sites. Quantification of sediment and oyster total PAH concentrations and RNA/protein expression will allow for both biomarker development and further understanding of the physiological consequences of exposure. Supported by the Northern Gulf Institute and NIUST NA07OAR4300494.

**TP027 Selection of a Surrogate MC252 Oil as a Reference Material for Future Aquatic Toxicity Tests and Other Studies** O. Pelz, BP, Gulf Coast Restoration Organization, BP Gulf Coast Restoration Organization, GCRO; J.S. Brown, Exponent Inc.; M. BenKinney, Exponent; P. Gardinali, Florida International Univ; C. Gong, BP; D.B. Grass, BP, GoM Thunder Horse PU; M. Huddleston, Cardno Entrix; G. Rand, Florida International Univ, Ecotoxicology & Risk Assessment; W. Stubblefield, Parametrix, Inc.; A.D. Ahnell, BP Gulf Coast Restoration Organization, Science,

Technology, Environment & Regulatory Affairs. Many scientific studies are planned that involve Macondo (MC252) crude oil; however, demand may exceed supply of the available source oil, such that surrogate crude oils are necessary. This presentation describes the process and analytical results for selecting an appropriate surrogate for MC252 oil. The selection criteria for surrogate candidates were based on a.) geography and oil family (also platform/well ownership), b.) crude oil chemistry and composition, and c.) aquatic toxicity testing. Oil produced from the BP facilities at Horn Mountain and Marlin satisfied the first criterion. Environmental forensic methods were then applied to several samples of different petroleum streams from these facilities to determine hydrocarbon profiles (including total petroleum hydrocarbons (TPH), volatile hydrocarbons (BTEX), saturated hydrocarbons (SHC – alkanes), polycyclic aromatic hydrocarbons (PAH), alkylated PAH, and geochemical biomarkers (steranes and triterpanes)). Based on the chemistry results, the two closest surrogate oils were selected and as a final criterion, laboratory-based acute and chronic toxicity tests are conducted on water accommodated fractions (WAFs) of these oils and MC 252 oil to enable direct comparisons of the aquatic toxicological properties (LC50 and EC10 values).

**TP028 Changes in Dissolved Hydrocarbon Content of Shallow Waters During and Shortly After the Deepwater Horizon Incident** A. Ross, C. Stalvies, C. Stalvies, X. Qi, E. Crooke, D. Fuentes, S. Armand, A. Revill, A. Talukder, CSIRO. The Deepwater Horizon incident began on April 20th 2010 and oil was released into the Gulf of Mexico until July 15th 2010. Mixing between the hydrocarbons and seawater while rising vertically about 1,500 meters to the surface, and mixing of the surface slick and seawater resulted in dissolution of components susceptible to partitioning into the aqueous phase. Such compounds include benzene, toluene, ethylbenzene, xylenes, naphthalenes and polyaromatic hydrocarbons. This presentation focuses on the dissolved hydrocarbon compounds and oil droplets detected and measured in the surface waters of the Gulf of Mexico during and shortly after the release. Between the 5th of June and 14th of September 2010 we investigated the abundance and distribution of these compounds using a prototype 3 fluorimeter hydrocarbon sensor array tuned to detect the presence of dissolved petroleum hydrocarbons in waters 1-2 meters below the sea surface. The Chelsea Aquatracker, Contros and Trios fluorimeters operated continuously while the survey vessel was underway. In total we surveyed over 8,998 linear nautical miles (~16,664line kilometers) and gathered over 2.2 million real time sensor data points. The survey area in the Northern Gulf of Mexico encompassed a large offshore geographic area from western Louisiana through to western Florida, including transits within a few miles of the Macondo well. These data are calibrated to 440 results from discrete near-surface water, and surface oil slick samples analyzed by gas chromatography mass spectrometry (GCMS). The technical details of the operation and geochemical calibration of this real time data stream are presented in accompanying posters. The results of this work will be presented to demonstrate the geographic distribution, and changes over time, of the dissolved hydrocarbon concentrations in surface waters the over the survey period. Associations of dissolved hydrocarbons and oil droplets with surface slicks will be illustrated, and presented with background data from baseline areas where no slick was ever detected.

**TP030 Acute Toxicity of the Oil Dispersant, Corexit 9500A, on Aquatic Photosynthetic Organisms** S. Johnson, RUTGERS Univ, Environmental Science; M. Gorbunov, RUTGERS Univ, Institute of Marine and Coastal Sciences. Dispersing oil is assumed to protect shorelines and aquatic organisms living within a few meters below the ocean's surface. Acute toxicity studies of the oil dispersant, Corexit 9500A, were evaluated using mysid shrimp, copepod, and fish. However information pertaining to the dispersant effects on the primary producers is lacking. The goal of this research was to assess the acute and chronic toxicity of Corexit 9500A on aquatic photosynthesis utilizing the fluorescence induction and relaxation technique. This fluorescence technique provides a comprehensive set of parameters that characterize the excitonic energy transfer in the light-harvesting antennae, the photochemistry in Photosystem II, and the electron transport to carbon fixation. The diatom, *Thalassiosira weissflogii*, (TW) and the zooxanthellae, *Symbiodinium* spp. (Zoox) were exposed to varying concentrations (.038 to 1.71 g/L) of Corexit 9500A. The mean 48hr EC<sub>50</sub> for the dispersant was .57 g/L and 1.33 g/L for TW and Zoox, respectively. At these concentrations, no effect to the photosynthetic electron transport on photosystem II acceptor side was detected. However, a marked decline in the quantum efficiency



of photosystem II and the energy transfer efficiency in the photosynthetic light-harvesting antenna was observed. The analysis revealed that Corexit 9500A adversely affects the primary photosynthetic reactions. This information can be utilized to organize dispersants by potency.

**TP031 The EPA Aquatic Life Benchmarks and Beyond: Improving Toxicity Assessments with Predictive Models and Empirical Data** S.R. Thoms, USEPA, Region 4, Superfund Division, USEPA, Region 4, Waste Division, Office of Technical Serv, USEPA, Region 4, Superfund Division, Superfund Support Branch, USEPA, Region 4, Waste Division, Office of Technical Serv; A.C. Bejarano, Research Planning Inc., Research Planning Inc.; U. Kipka, Anchor QEA. Estimating the bioavailability and toxicity of mixtures of crude oil chemicals is critical in assessing the impact of an oil spill. The water-accommodated fraction (WAF) of the oil is often tested to estimate potential toxicity if oil were released to receiving waters. The Operational Science Advisory Team (OSAT) extracted WAF from three types of residual oil collected at four case study locations, one within each impacted State. OSAT used the toxic unit approach, derived from the EPA aquatic life benchmarks, to assess potential toxicity of PAH mixtures to aquatic organisms, where the sum of PAH toxic units that exceeded unity may indicate potential adverse effects. For certain WAF samples PAH toxic units exceeded unity. The lack of actual WAF toxicity testing and field-collected chemistry data for realistic exposures, where dilution would occur, created controversy among the OSAT with regard to interpretation and presentation of the data. Many assumptions went into the development of the EPA aquatic life benchmarks. The additive model of PAH toxicity on which benchmarks were based is only as good as the description of toxicity and bioavailability of each chemical. However, predictive models are also available and should be evaluated to better assess the toxicity of oil residues to aquatic fauna when empirical data is limited or unavailable. This work aims to assess the current capability of both single parameter, octanol-water partitioning linear free energy relationships (LFERs) and polyparameter LFERs (pp-LFER) to predict literature-derived 95% species protection levels (HC5). Assumptions made in the derivation of the EPA aquatic life benchmarks will be examined. A pp-LFER model for coal tar more or less predicted the partitioning of PAHs between residual oils and WAF, while the single-parameter LFER used to derive the EPA aquatic life benchmarks overestimated solubility. Implications of observed deviations in the literature toxicity information or the WAF-derived bioavailability results from model assumptions will be discussed in terms of how model fits affect the strength and reliability of toxicity predictions.

**TP032 The Interaction Effects of Natural Oil and Oil Dispersants with Delivery System on Embryos of *Palaemonetes pugio*** J. Rayburn, Jacksonville State Univ, Dept of Biology. Grass shrimp (*Palaemonetes pugio*) are an important species from an ecological perspective because they serve as a link for energy transfer between trophic levels in the coastal food web. Grass shrimp are predators of meiofauna and small infaunal polychaetes, oligochaetes and nematodes and are consumed in large quantities by commercially important fishes and forage species. As part of the overall effort in determining the effects of hydrocarbon on estuarine environments in the Gulf of Mexico, this proposal would investigate the effects of oil and oil dispersant toxicity to grass shrimp (*Palaemonetes pugio*). The question investigated was to determine the acute toxicity of both the oil, the oil dispersants used and if there are synergistic effects between the two. The grass shrimp were collected and identified to species (*Palaemonetes pugio*), sexed, length, reproductive status, (state of embryos if present) and observations of parasites recorded. Ten females and 15 males were kept in glass aquaria (approximately 35 liters) at 20 ppt sea water (made with artificial sea salts). Grass shrimp were maintained in standard laboratory conditions, 12 hr light dark, 27 degrees C. The grass shrimp were feed high protein fish food. Gravid females were removed and ages of embryos were determined. Embryos were collected at tissue cap stage and 3 days prior to hatch. Embryos were placed in individual 24 well plates with 2 mls of test solution. Twenty-four wells (one plate) were used for controls and for each test concentration. Embryos were observed daily for mortality. Embryos were exposed to a concentration series of oil, oil dispersants and mixtures of the two to determine the LC50 using probit analysis. Toxic unit analysis was performed to quantify the interactions. This study further validates the use of grass shrimp embryos in assessing the impact of chemicals and mixtures in estuarine environments.

**TP033 Development of a Rapid Method for the Analysis of Olafactory Compounds and Petroleum Hydrocarbons in Seafood Using SPME-GC-MS** S. Genualdi, Environment Canada, Science And Technology Branch, Environment Canada; L. DeJager, T. Begley, FDA. During the aftermath of the Deepwater Horizon oil spill in April 2010, NOAA and the FDA revised the guidelines for re-opening closed seafood harvest areas. In order to improve the response time in the event of future oil spills, a rapid method using a 5975T transportable GC/MS is being developed for olfactory compounds indicative of petroleum taint. This would increase sample throughput and replace the currently required human sensory panel. The olfactory compounds used in this study consist of benzene, ethylbenzene, toluene, p-xylene, indan, tetralin, mesitylene, naphthalene, and 1-methyl naphthalene. Petroleum hydrocarbons were included as analytes of potential interest and consist of straight chain, branched, and cyclic alkanes. The analytical method using solid phase microextraction (SPME) gas chromatography – mass spectrometry (GC-MS) was first optimized using aqueous solutions. This method was further tested on seafood (finfish, shrimp, and mussels) obtained from local markets and also on seafood fortified with olfactory compounds, petroleum hydrocarbons and oil from the Deepwater Horizon Spill. This automated method significantly reduces analysis time while providing a transportable option to obtain quantitative results at concentrations < 50 ppb in the field in case of a future disaster.

**TP034 Analysis of Polyaromatic Hydrocarbons (PAH) in Seafood Using HS-SPME-GC-MSD** L.S. Delager, T. Begley, Food and Drug Administration, Center for Food Safety and Applied Nutrition. Polyaromatic Hydrocarbons (PAHs) are a class of organic pollutants which are found in significant quantities in crude oil. In addition to the primary PAH structures, mono, di and tri alkyl substituted PAH moieties have been identified. PAHs contamination is a concern because some of these compounds have been shown to have significant gene toxicity. The recent Deep Water Horizon oil spill contaminated large areas of federal and state fishing waters. According to federal and state protocol, before the fisheries could be reopened seafood samples must pass sensory testing for taint and then pass chemical analysis for PAHs. Because of the low concentration of these analytes and the complexity of the tissue matrix, most analyses require pre-concentration and clean up prior to introduction onto analytical instrumentation. Classic extraction techniques, such as liquid-liquid extraction and solid phase extraction, are time-consuming and require significant quantities of solvents and consumable supplies. In order to reduce analysis time and decrease analysis costs it is desirable to develop an automated method for the analysis of PAHs and PAH homologs in seafood samples. Solid phase microextraction (SPME) is a sorptive extraction technique that can be used in conjunction with GC-MS to provide automated sample preparation for volatile and semi-volatile analytes. This technique has been used for the analysis of low molecular weight PAHs in seafood, but has not been applied to the higher molecular weight (and less volatile) PAH moieties. A SPME-GC-MS method for the determination of 16 PAHs and 40 alkyl substituted PAH compounds in finfish, oysters and shrimp has been developed. Parameters optimized include fiber coating, extraction time, extraction temperature, sample size and sample composition. Significant carryover of high molecular weight PAHs was problematic, but this was overcome by using a 7µm PDMS fiber and a high injection port temperature. Using a 3 hour extraction time, we achieved limits of detection of less than 10 ppb in seafood which are lower than the concentrations outlined in the reopening protocols. This method will provide an important analytical tool for rapid response in the event of another oil spill.

**TP035 Natural Oil and Gas Occurrences in the Mississippi Canyon Protraction Area: Geochemistry and Origin of Naturally Occurring Hydrocarbons in Sediments** A. Kornacki, Weatherford, BP GCRO, 1315 West Brooklake; P.D. Carragher, BP Gulf Coast Restoration Organization, Science, Technology, Environmental and Regulatory Affairs. Sediments near natural petroleum seeps contain mixtures of fresh oil, biodegraded oil, thermal and/or microbial natural gas, and recent organic matter (ROM). We applied quantitative methods to separate the relative contribution of these components in >100 deepwater sediment samples pre-dating the DWH incident, and present a model integrating the data and relevant processes. Deepwater Gulf of Mexico (GOM) sediments generally contain small amounts of organic matter -- the debris from the marine and terrestrial biospheres: e.g., algal or plant material. Turbidity currents transport waxy plant material into the deepwater GOM. This type of ROM consists largely

of  $C_{23}$ - $C_{31}$  n-alkanes with an odd carbon-number preference, typically increasing in concentrations to a peak at  $C_{29}$ . Crude oil is different: n-alkanes lighter than  $C_{20}$  are more abundant than  $C_{20}$ , n-alkanes;  $C_{23}$ - $C_{31}$  n-alkane concentrations decrease with increasing C number, and do not exhibit an odd carbon-number preference. Fingerprinting the oil in seeps is challenging because ROM also contains some biomarkers present in crude oil. We used a geochemical parameter based on the abundance of n-alkanes lighter and heavier than  $C_{23}$  to estimate the relative amount of crude oil vs. ROM in seep samples. The LHAR (*Light to Heavy Alkane Ratio*) value is higher in seep samples enriched in oil than in seep samples enriched in ROM. A map of the Mississippi Canyon Protraction Area and vicinity shows the complexity of the oil seep system.

**TP036 Natural Oil and Gas Seeps and Oil Slicks in the Mississippi Canyon Protraction Area: Remote Sensing by Satellite, Surface Monitoring and AUV Surveys** C. Scherschel, BP, BP, 1315 West Brooklake; R. Frost, BP, Upstream Technology, 1315 West Brooklake. Satellite data has proven very useful in the detection of oil slicks at sea, both from naturally occurring oil seepage, and the variety of human activities that cause oil releases. Repeat satellite passes, often over a number of years, identify areas of persistent release that are clearly from natural seepage. In detail, this process requires quality control of the data set to ensure the correct weather conditions for interpretation are met. This poster presents preliminary results of a project to identify oil slicks of a natural origin in the vicinity of the Mississippi Canyon Protraction area. The time period covered is the period up to April 2010, predating the DWH Incident and the resulting oil slick. The linkage of these natural slicks to seabed features and the sub-seafloor geology will be shown. Autonomous Underwater Vehicles (AUVs) provide detailed and accurate data on the seafloor in deepwater environments. The physical features of the seabed can be mapped with resolutions of 1 meter or less over areas in the order of 100 km<sup>2</sup>. In addition, sub-bottom profiling provides data on the stratigraphy of near-seabed sediments. This presentation focuses on the identification of natural seeps on the seafloor, in both map and cross-sectional view, over a large area surrounding the Macondo well in block Mississippi Canyon 252. The poster will show the geological context for an area of prominent seep features, tracing the seeping hydrocarbons from their migration pathway through the sedimentary sequence up to the seabed and then to the water surface. This data provides the local context for the sediment sampling work that was reported by the OSAT in December 2010; and the sediment fingerprinting paper presented by Brown et. al. at the SETAC special session in April 2011. At selected sites, the AUV performed a more detailed photomosaic survey, examples of which will be presented in this poster.

**TP037 Time Integrative Large Volume In Situ Water Extraction for Total and Dissolved Trace Organics** B. Hepner, Aqualytical Services Incorporated; D. Mabe Jr., C.I.Agent Storm?Water Solutions. The standard grab sample water bottle has a volume of 1 liter or less. This is an insufficient volume for the analysis of many contaminants at the sub-microgram per liter concentrations necessary for assessing compliance with water quality guidelines. In principle, the level of detection could be improved by collecting a larger volume, time integrative sample. The difficult task in concentrating large volume samples is capturing the pollutants in both the particulate and dissolved phases without allowing significant break-through of the contaminants. In order to accomplish this, two different pollutant removal mechanisms must be employed. Pollutants bound to the particulate phase can be removed via a filtering system that physically removes all particulate matter. Those pollutants in the dissolved phase, however, must be extracted from the water utilizing a substance that sequesters them. In order to extract in situ large volumes of water while separating the pelagic sediments from the dissolved fraction, a two stage Luer locked disk system coupled to a small submersible pump was developed. The first stage disk used lofted glass depth filtration to quantitatively retain pelagic sediments for extraction and analysis. The second stage disk sequestered dissolved trace organics of interest, with solid phase extraction media. The small submersible pumping system would draw water slowly through the disks at 10-50 ml/min. providing a time integrative extraction event, representing days to weeks, and up to 100 liters of water. GC/MS/ LVI results from two studies will be presented using this submersible extraction system: An ultra low level PAH study, comparing its relative concentration in the pelagic sediments to its associated dissolved water concentration from a large volume extraction event of 50 to 100 liters. The second is a study of the water column off

Dauphine Island, Alabama, which was continuously monitored using this extraction system for PAHs in the PPT level during the Horizon spill event. This provided three months of monitoring before and during the event, which will also be presented.

**TP038 Productivity of Colonial Waterbirds and Seabirds Breeding in the Northern Gulf of Mexico Following the Deepwater Horizon Accident** J.R. Wakefield, P. Reilly, Cardno Entrix. In 2011, BP undertook one of the largest efforts implemented to date to estimate productivity (fledglings produced per active nest) of colonial nesting waterbirds in the northern Gulf of Mexico. Ground and aerial surveys were used to gather productivity data at 40 nesting colonies. Thirty of these colonies, all in eastern Louisiana, were stratified into three categories based on the peak level of oiling recorded in surrounding habitat during the summer of 2010. (The 10 remaining colonies were located along north Texas shorelines, which were unaffected by the Deepwater Horizon accident.) Nest counts began April 15, 2011 and continued through July 1, 2011. Ground counts occurred every three days and aerial counts and photo-documentation occurred every 15 days. Focal species included brown pelican, great egret, black skimmer and laughing gull. Other species, such as royal tern, Caspian tern, least tern, sandwich tern, and American oystercatcher, were included as field conditions dictated. Species-specific data describing the productivity of individual colonies will be presented along with statistical evaluations of the effect of 2010 oiling on 2011 productivity. These data will be supplemented with data from the ongoing satellite tracking of brown pelicans and great egrets to present a detailed picture of birds in the northern Gulf of Mexico one year following the Deepwater Horizon incident.

**TP039 Contamination Profiles of PCB Congeners, Chlorinated Pesticides and PBDEs in Sediment and Fish Samples from Coastal Waters Off Savannah, Georgia, USA** B.M. Cassidy, Murray State Univ, Chemistry and Watershed Studies Institute; P. Chakraborty, J.P. Richardson, K.S. Sajwan, Savannah State Univ, Natural Sciences and Mathematics; B.G. Loganathan, Murray State Univ, Dept of Chemistry & Watershed Studies Institute. Classical persistent organic pollutants and emerging new organic pollutants are of great concern due their negative effects on environment, wildlife and human health. Estuaries and coastal marine environments are considered the most sensitive areas to the accumulation of these pollutants. Objective of this study was to understand the contamination status of classical and emerging pollutants in the coastal waters off Savannah, Georgia. PCB congeners, chlorinated pesticides and PBDEs were analyzed in sediment and various fish species samples collected from riverine (Vernon River, Harmon Canal) and coastal waters off Savannah, Georgia. Standard analytical procedures were followed to determine the concentrations of these contaminants. Results revealed that detectable levels of PCBs, pesticides and PBDEs were found in sediments and fish samples from this region. The results were compared with the earlier studies on these pollutants to discern temporal trends.

**TP040 Distribution and Air-Sea Exchange of Current-use Pesticides (CUPs) from East Asia to the Arctic** G. Zhong, Helmholtz-Zentrum Geesthacht, Yantai Institute of Coastal Zone Research, CAS; Z. Xie, Helmholtz-Zentrum Geesthacht, Helmholtz-Zentrum Geesthacht, Dept for Environmental Chemistry, Helmholtz-Zentrum Geesthacht, Institute of Coastal Research, Dept for Environmental Chemistry, GKSS Research Centre, Research Scientist; M. Cai, Polar Research Institute of China; A. Moeller, R. Sturm, Helmholtz-Zentrum Geesthacht; J. Tang, Yantai Institute of Coastal Zone Research, CAS; G. Zhang, Guangzhou Institute of Geochemistry, CAS; J. He, Polar Research Institute of China; R. Ebinghaus, Helmholtz-Zentrum Geesthacht. Surface seawater and boundary layer atmospheric samples collected on the ice-breaker *M/V Xuelong (Snow Dragon)* from the East China Sea to the high Arctic (33.23–84.5 °N) in July to September 2010 were analyzed for six current-use pesticides (CUPs) trifluralin, endosulfan, chlorothalonil, chlorpyrifos, dacthal and dicofol. Particulate CUPs in both air and seawater is minor. All CUPs were not only detectable in all oceanic air, but also had levels as high as 100s pg/m<sup>3</sup> in Japan Sea. Gaseous CUPs basically decreased sharply from East Asia (36.6 and 45.1°N) to the north, following a relatively slow descent until it bottomed out at the Bering Sea (58.1-66.4°N), thereafter air CUPs more or less mounted up again in Chukchi Sea. The dissolved CUPs ranged widely from α-endosulfan, chlorpyrifos and dicofol in seawater were roughly consistent with their latitude trends in air. Trifluralin in seawater was relatively high at Japan Sea (35.2°N) and evenly distributed from 36.9 to 72.5°N, whereas

below detection limit in the highest latitudes in Chukchi Sea. In contrast with other CUPs, chlorothalonil and dachthal in Chukchi Sea were far more abundant than those in East Asia. The air-sea gas exchange flux of CUPs was generally dominated by net deposition.

**TP041 The Global Distribution of Perfluorinated Compounds and Long-range Transport via Marine Water in the Arctic, Atlantic Ocean and Antarctic Coast**

Z. Zhao, Helmholtz-Zentrum Geesthacht, Dept for Environmental Chemistry; Z. Xie, Helmholtz-Zentrum Geesthacht, Helmholtz-Zentrum Geesthacht, Dept for Environmental Chemistry, Helmholtz-Zentrum Geesthacht, Institute of Coastal Research, Dept for Environmental Chemistry, GKSS Research Centre, Research Scientist; A. Moeller, Helmholtz-Zentrum Geesthacht; R. Sturm, Helmholtz-Zentrum Geesthacht, Max-Planck Str.1; R. Ebinghaus, Helmholtz-Zentrum Geesthacht. Since the ubiquitous occurrence and potential toxicity to human beings, the perfluorinated compounds (PFCs) have attracted more concerning in the recent years. In 2010, one of the PFCs, perfluorooctane sulfonate (PFOS) was added to Annex B of Stockholm Convention on Persistent Organic Pollutants (POPs), which means the official ban in the whole world. However, the emission of some other PFCs from the manufactures and products are still potential threats to the environment and living creatures. Perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFSAs) are very soluble, and the PFOS reside in marine surface water recently was estimated similar to the estimated release amount. The persistent PFCs could be transported via ocean current to polar areas which results the occurrence in polar water and biota. In this study, the global distribution and long-range transport of PFCs were investigated in marine surface water. 24 PFCs were detected in 78 seawater samples from Antarctic, Atlantic and Arctic area collected in 2010-2011. The levels were compared to former studies to figure out the trend of PFCs release to the environment. The dynamics of long-range transport of PFCs were investigated and the fates of different compounds were predicted.

**TP042 Regional Differences of Concentration and Chiral Signatures of DDTs and HCHs in the Yellow Sea**

S. Hong, Korea Ocean Research & Development Institute, Oil and POPs research group, Korea Ocean Research & Development Institute; W. Shim, U. Yim, Y. Jin, S. Ha, Korea Ocean Research and Development Institute; G. Han, Korea Ocean Research and Development Institute, Oil & POPs Research Group; N. Kannan, Korea Ocean Research and Development Institute. Yellow Sea is a semi-enclosed body of water bound by Chinese mainland to the west and Korean Peninsula to the east. Yellow Sea has been threatened by both land- and sea-based sources of pollution resulting from the extensive economic development in the coastal zone. DDTs and HCHs are some of major contaminants of concern in this region. To assess their contamination characteristics, we collected air, seawater, and sediment samples from coastal and offshore regions and conducted chemical analysis. Generally, China showed a relatively high level of DDTs and HCHs at most matrixes, which could be due to the massive production and extensive use of these chemicals in China in the past. In order to understand the chiral signature of DDT and HCH compounds in this water body, chiral analysis for *o,p'*-DDT and *a*-HCH was conducted. In the Yellow Sea, (+) *o,p'*-DDT was more abundant than (-) *o,p'*-DDT. The portion of the (+) enantiomer in the sediment is relatively enriched. Generally, the chiral signature of *o,p'*-DDT in air of the Yellow Sea was closer to that in China in winter compared to Korea. But, in summer, it resembled the Korean sources. This indicates clearly that the chiral signature of *o,p'*-DDT in the Yellow Sea is mainly influenced by the direction of the wind. On the other hand, *a*-HCH was racemic in most of samples.

**TP043 Dietary Exposure of Juvenile Whitemouth Croaker to PBDEs: Uptake, Biotransformation and Depuration Kinetics**

M.C. Pieroni, J. Leonel, G. Fillmann, Universidade Federal do Rio Grande. Polibrominated Biphenyl Ethers (PBDEs) are flame retardants compounds widely used in plastics, textiles, furniture, electronics, automobiles, and many other materials. Although PBDE are persistent and resistant to degradation, earlier studies have established that some fish can debrominate certain PBDEs congeners. Carps, for example, readily debrominate BDE 99 to BDE 47 while salmon slowly debrominate BDE 99 to BDE 49. Therefore, this study aimed to understand the accumulation and depuration kinetics of PBDEs in the whitemouth croaker (*Micropogonias furnieri*), an important species in the Brazilian fishery industry, as well as its ability to debrominate both BDE 99 and BDE 153 to lighter congeners. Approximately 60 juveniles of

whitemouth croaker, divided in two 250 L tanks, were used for each exposition (BDE 99, BDE 153 and control). BDE 99 and BDE 153 were chosen due to its use in the pentaBDE technical mixture, which is inferred as the most used mixture in Brazil according to the environmental data. They were exposed to artificially contaminated food for 30 days (50ng/dia/peixe of PBDEs) followed by non-contaminated food for others 30 days. Sampling, for both control and contaminated tanks, occur on days 0, 1, 5, 10, 15 and 30 (contamination period) and on days 41 and 60 days (depuration period). After anaesthesia with benzocaine fish size and weight were recorded and blood were collected. Liver, muscle, guts, stomach and feces are sampled to determined PBDEs concentrations.

**TP044 Microbial Transformations of Pollutants in Arctic Ecosystems**

C. Larose, S. Cecillon, T.M. Vogel, Ecole Centrale de Lyon, Environmental Microbial Genomics Group, Laboratoire Ampère. The Arctic environment is undergoing changes due to climate shifts, long-range transportation of contaminants and increased human activity. That the Arctic is polluted by industrial processes was recognized several decades ago, serving in part as a major impetus for funding Arctic research and highlighted the role of atmospheric transport in contaminating isolated environments with few local pollution sources. Once deposited, these contaminants can undergo a variety of transformations that are biotically and abiotically mediated before contaminating the food chain. Snowpacks and aquatic ecosystems are involved in contaminant cycling and may influence their fate in Arctic systems. Among the different pollutants, this work focuses on mercury as well as legacy and emerging persistent organic pollutants (POPs) in Arctic ecosystems, with emphasis on microbial transformations and metabolism. Snow and fjord water samples were collected at different spatial scales from an Arctic field site, Ny-Ålesund, Norway (79°N). Bacterial diversity and community structure were measured by ribosomal intergenic spacer analysis (RISA) and phylogenetic microarrays. Some identified functional genes involved in POP and mercury metabolism were measured by quantitative polymerase chain reaction analysis (qPCR) and results were linked to contaminant levels. This preliminary work will provide a better understanding of biotic contaminant degradation pathways in arctic environments.

**TP045 Evaluation of EROD Activity as a Biomarker of PAHs Exposure in Estuarine Fish and Crustacean in Southern Brazil**

A. Machado, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas; I.F. Barcarolli, C.L. Paganini, T. Martins Lopes, S. Carvalho Rodrigues, Universidade Federal do Rio Grande; A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. EROD (7-ethoxyresorufin-*O*-deethylase) activity has been widely used as a biomarker of exposure to organic compounds, such as biphenyls, polychlorinated, and polycyclic aromatic hydrocarbons (PAHs). However, few studies focus the application of EROD data to predict environmental health. The aim of this study was to evaluate EROD activity as a biomarker of PAHs contamination in two species from different trophic levels, the fish *Micropogonias furnieri* and the blue crab *Callinectes sapidus*. Animals were collected seasonally in different sites of the Patos Lagoon estuary (Southern Brazil) with different levels of contamination. EROD activity and PAH concentrations were measured in fish liver and crab. To determine its suitability as a biomarker, EROD activity data was correlated with tissue PAH concentration, biotic and abiotic (water) parameters. The higher mean values of EROD activity ( $\mu\text{mol resorufin} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ) were observed in winter for fish (245.0) and in autumn for crab (325.4), while the highest mean values of PAHs concentrations ( $\text{ng} \cdot \text{g}^{-1} \text{ ww}$ ) were found in winter for both fish (173.8) and crab (361.6). Twenty-five aromatic compounds of different molecular weight, an indication of the number of aromatic rings, were identified in fish liver and crab hepatopancreas. For high molecular weight PAHs, linear regression analysis showed that fish liver concentrations higher than  $28.22 \text{ ng} \cdot \text{g}^{-1} \text{ ww}$  were strongly and positively correlated with tissue EROD activity. In the other hand, no correlation was observed for low molecular weight compounds and EROD activity in fish liver. Also, no significant correlation was observed in crab hepatopancreas for either high or low molecular weight PAHs. Significant correlations between EROD activity and abiotic parameters such as water temperature and pH were also observed. These findings suggest that these parameters influence the biotransformation of xenobiotics. Taken altogether, findings described in the present study suggest the potential suitability of EROD activity as a reliable biomarker of exposure to high molecular weight PAH exposure in estuarine fish in different seasons. [Supported by Brazilian CNPq (CT-Hidro) and CAPES (Ciências do Mar)].



**TP046 Chronology of Chemical Loadings to Tidal Marshes in the Delaware River Estuary** M.W. Schafer, Academy of Natural Sciences, Staff Scientist, Patrick Center for Environmental Research, Academy of Natural Sciences, Staff Scientist; D.J. Velinsky, Patrick Center for Environmental Research, Academy of Natural Sciences; J.T. Ashley, Philadelphia Univ; C. Sommerfield, College of Marine and Earth Studies, Univ of Delaware; R. Greene, Delaware Dept of Natural Resources and Environmental Control; T. Fikslin, Delaware River Basin Commission. During the early 20th century, there was a substantial loading of chemical contaminants, such as PCBs, PAHs and DDT throughout the Delaware River Estuary. Since the mid-to-late 1980s, inputs of various "emerging" chemicals, e.g., PBDEs, have also increased. However, since the early 1960's, there have been efforts to reduce chemical loadings, improve ecological conditions within the estuary and reduce the concentration of contaminants in finfish. Sediment cores were taken from ~30 tidal freshwater and estuarine marshes in the Delaware River Estuary to estimate historic loadings of chemical contaminants, nutrients and their potential ecosystem impacts. Chronologies were determined with  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  isotopes. A preliminary analysis suggests an average sediment accumulation rate ( $^{210}\text{Pb}$ ) of  $0.62 \pm 0.24$  cm/yr (12% RSD;  $n=22$ ) with a range of 0.22 to 1.3 cm/yr. Sediment-bound PCBs and other organic contaminants increased in concentration starting in the late 1930s to mid-1950s. Many organic contaminants showed peak concentrations in the 1960s to 1970s. There were also river basin specific differences in contaminant concentrations presumably related to changes in watershed land use. PCB and other sediment contaminant inventories were highest in more urban watersheds. The benefits of coring and developing accurate sediment contaminant chronologies are key to reconstructing historic anthropogenic impacts on the environment. This information will be used to evaluate current environmental conditions and guide future restoration efforts.

**TP047 Microplastics in Korean Beach: Spatial Distribution, Composition, and Associated Organic Contamination** S. Hong, Korea Ocean Research & Development Institute, Oil and POPs research group, Korea Ocean Research & Development Institute; W. Shim, G. Han, K. Heo, S. Ha, Korea Ocean Research and Development Institute. Currently, marine debris is of global environmental concern. A large portion of marine debris consists of plastics. Plastic materials that were discarded, disposed or abandoned at sea are broken down to microplastics through natural weathering processes such as degradation under UV radiation and abrasion by wave action. Microplastics enter marine environment by other means such as sea transportation or in industrial accidents. The microplastics are capable of adsorbing organic contaminants from water column acting as a useful monitoring tool for marine pollution. In order to understand the contamination status and characteristics of microplastics pollution in Korea, we conducted a beach monitoring survey and subsequently chemical analysis. The density of plastic debris was high at the upper tidal zone. < 5mm size plastics were dominant. Plastic fragments and fibers less than 1mm were also abundantly observed. Interestingly, expanded polystyrene (EPS) was the major microplastics of the beach. The average number of microplastics on the beach was 1300 EPS and 16 pellets per m<sup>2</sup>. This is primarily due to the extensive use of EPS buoy in the aquaculture in Korea. EPS distribution was more at the upper tidal zone than plastic pellets. Persistent organic contaminants such as PCBs, DDTs, and PAHs were detectable in both EPS and the pellet. The POPs concentration was relatively high in pellets on an individual basis (greater surface area and/or active adsorption site) but the overall contribution from EPS was greater due to its high density of availability in a typical Korean beach. In general, aged EPS and pellet accumulated more of chemical residues than fresh ones.

**TP048 Sponges as Biomonitors of PHA Contamination in Rio de Janeiro, Brazil** A. Wagener, Pontificia Universidade Católica do Rio de Janeiro, Química; K. Tellini, Pontificia Universidade Católica do Rio de Janeiro; A.H. Nudi, Pontificia Universidade Católica do Rio de Janeiro, Chemistry; D. Batista, A. Scofield, C. Sette, N. Floriano, Pontificia Universidade Católica do Rio de Janeiro. Filter feeder organisms have been used to monitor oil contamination in coastal and estuarine waters. The present study evaluates the potential of marine sponge *Hymeniacidon heliophila* as biomonitor of PAH contamination in Rio de Janeiro, Brazil. Samples (sponges and waters) were collected in August 2010 from three areas in the polluted Guanabara Bay as well as in one reference site (Itaipu). Additionally, *Perna perna* was sampled for compare with *H. heliophila* since the mussels are widely considered efficient biomonitors. The determination of 16 PAH

(USEPA) and its alkylated homologues were obtained by GCMS. The results show high correlation between species for  $\Sigma 16\text{PAH}$  ( $r=0.68$ ) and the inter-specific total mean concentration did not vary significantly (ANOVA;  $p < 0.05$ ). The highest mean concentration of  $\Sigma 16\text{PAHs}$  in sponges was observed in the most polluted site (1428 ng g<sup>-1</sup>; Praça XV), followed by Urca (348 ng g<sup>-1</sup>). Benzo[a]pyrene concentration ranged from 10 ng g<sup>-1</sup> (Itaipu and Marina) to 139 ng g<sup>-1</sup> (Praça XV). Unexpectedly, sponges from the reference site showed higher concentration of  $\Sigma 16\text{PAHs}$  (193 ng g<sup>-1</sup>) when compared a marina site located inside the bay (Marina da Glória; 174 ng g<sup>-1</sup>). This area recently became affected by discharge at tons of contaminated sediment from the Rio de Janeiro Harbor and, probably, the activity results in contamination of local ecosystems. As suspension feeders, these animals have high retention rates for particles from 0.2 to 50  $\mu\text{m}$  and consequently can collect pollutants from both suspended and dissolved phases. *Hymeniacidon heliophila* has a response very similar to mussel *Perna perna* and their distributions from North Carolina (USA) to Santa Catarina (Brazil) make them a good alternative for biomonitoring programs in the coastal shallow waters of Western Atlantic. Financing by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

**TP049 Development and Evaluation of a Multimedia Environmental Fate Model for Phthalate Esters in a Marine Aquatic Food Web** G. Zandpour, H. Lai, Simon Fraser Univ, Biological Sciences; M.G. Ikonomou, Dept of Fisheries and Oceans, Ocean Chemistry; F.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment). Di-phthalate esters (DPEs) are widely used commercial chemicals. Their main application is as plasticizers. DPEs are high volume production substances that have been observed to be present in environmental media from many locations around the world. There is concern over the potential impacts of di-phthalate esters and their main metabolites, the mono-alkyl phthalate esters (MPEs), in wildlife and humans. This concern is partly caused by the lack of a quantitative understanding of the environmental fate of these chemicals in the environment. To improve the understanding of the relationship between the release of DPEs into environment and resulting concentrations of DPEs and MPEs in abiotic and biotic media, we developed and tested a multimedia environmental fate and food web model to describe the distribution and transformation of DPEs and MPEs in an aquatic ecosystem. To test the model, we conducted a field study of the distribution of DPEs and MPEs in a marine Inlet in Vancouver, Canada. In this presentation, we present the model, describe the field study and discuss the performance of the model. We demonstrate that the calculated sediment water partition coefficients (organic carbon normalized) are in good agreement with the corresponding independently measured values for both DPEs and MPEs. The only exception was dimethyl phthalate (DMP). Both model calculated and observed bioaccumulation factors of DPEs and MPE in fish and benthic invertebrate species are also presented. The model was applied to assess the combined loadings of DPEs into the Inlet and to assess the real-world half-life time of DPEs and MPEs in an aquatic environment. The multimedia mass balance environmental fate model in conjugation with a food web model provides a better understanding of the distribution and transformation of DPE in aquatic ecosystems. The model can be used in other aquatic environments, with their own system specific and chemical specific parameters. The model is expected to be useful for preliminary ecological risk assessment in order to predict exposure concentrations, internal body burdens and possible remediation targets.

**TP050 Inputs of Antifouling Paint-derived Dichlorodiphenyltrichloroethanes (DDTs) to a Typical Mariculture Zone (South China)** H. Yu, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; H.f. Cheng, E.Y. Zeng, Guangzhou Institute of Geochemistry. Existing evidence indicated that dichlorodiphenyltrichloroethane (DDT)-containing antifouling paints were an important source of DDT residues to mariculture zones. However, the magnitude of the impact on aquafarming environment has remained largely unknown. In the present study, the concentrations of DDT and its metabolites (designated as DDXs) were determined in harbor sediment and antifouling paint samples collected from a typical mariculture zone in South China. Compositional and concentration correlation analyses implicated the DDT-containing antifouling paints for fishing boat maintenance as an important source of DDT in the mariculture zone. The annual emission of DDXs to the study region was estimated at 0.58 tons/yr. Furthermore, a comparison of the expected DDT loadings in pelagic fish and field measurements indicated that fish feed especially trash fish was a major

source of DDTs in the fish body. Nevertheless, the use of DDT-containing antifouling paints should be limited to prevent further deterioration in aquaculture environment.

**TP051 Accumulation and Depuration of Polycyclic Aromatic Hydrocarbons (PAHs) in Benthic Gastropods and Crabs: Field and Laboratory Studies** Y. Goto, K. Uehara, H. Nakata, Kumamoto Univ. It has been reported that there are significant sources of PAHs in coastal regions of the Ariake Sea and Yatsushiro Sea, western Japan. High concentrations of PAHs were detected in sediments from both areas, at the levels of several tens of mg/kg dry wt. basis. However, little information is available on the contamination of PAHs in organisms. In this study, we have analyzed 17 PAHs in gastropods (*Nassarius festiva*) collected from four locations of the Ariake Sea and Yatsushiro Sea to understand the status of contamination of PAHs in those regions. PAHs were also analyzed in crabs (*Macrophthalmus japonicus*) to compare the residue levels of PAHs between benthic species. PAHs were detected in all samples analyzed in this study. High concentrations of PAHs were found in gastropods from the Omuta River (Ariake Sea) and Tanoura Bay (Yatsushiro Sea), at the average levels of 102 ng/g and 82.6 ng/g (wet wt.), respectively. The residue levels of PAHs in samples of both sites were more than one order of magnitude greater than those in other reference sites. Elevated concentrations of PAHs were also detected in crabs from Tanoura Bay. Fluoranthene and pyrene were dominant components among PAH congeners in both species. Interestingly, high molecular PAH congeners, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and indeno[1,2,3-*cd*]pyrene were predominant in gastropods from Tanoura Bay, which is probably due to discharge of wastewater from a chemical factory producing carbon materials in this bay. In this study, depuration of PAHs was examined in gastropods and crabs to evaluate their potentials for degradation/metabolism of PAHs. The crabs and gastropods collected from Tanoura Bay were transplanted to water tanks containing unpolluted artificial seawater to depurate PAHs in their bodies. The concentrations of PAHs in transplanted crabs drastically decreased from 396 ng/g (wet wt.) to 10.2 ng/g during a four-week experiment in the artificial seawater tank. Significant correlations of PAHs concentrations were found between sediments and crabs. The decline of PAH residues was also observed in gastropods in an artificial seawater tank. These results suggest that organic particles in sediment may play an important role to accumulate/depurate PAHs in the benthic organisms.

**TP052 Brain Region and Tissue Distribution and Patterns of Bioaccumulative Perfluoroalkyl Acids (PFAAs) in Highly Exposed East Greenland Polar Bears** A. Greaves, Carleton Univ, Dept of Chemistry; R.J. Letcher, National Wildlife Research Center, Carleton Univ, Wildlife Toxicology Research Section, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada; C. Sonne, R. Dietz, National Environmental Research Institute, Aarhus Univ, Dept of Arctic Environment; E.W. Born, Greenland Institute of Natural Resource. Perfluorinated sulfonates (PFSAs) and carboxylic acids (PFCAs) of varying chain lengths are perfluoroalkyl acids (PFAAs), which have been shown to bioaccumulate in wildlife and humans. Accumulation of PFAAs in wildlife has been largely thought, and in some cases demonstrated (e.g., perfluorooctane sulfonate (PFOS)), to be protein-association driven, although transport, deposition and accumulation of PFAAs in the body is not well understood. Previous studies have largely assumed that the liver is the major repository of accumulated PFAAs, and thus distribution of these compounds in other areas of the body is virtually unknown. Without investigating other regions of the body, it is difficult to interpret the overall PFAA pharmacokinetics, and thus tissue-/compartment-specific exposure to PFAAs in wildlife. Polar bears (*Ursus maritimus*), being the apex predator in the Arctic marine ecosystem, are an ideal species for studying contaminants in the Arctic. This study focuses on the distribution and preferential accumulation of PFAAs in specific regions of the brain as well as tissues (e.g., liver, muscle and fat) and plasma in highly PFAA exposed East Greenland polar bears. One hypothesis that we tested was that PFAAs will accumulate specifically and preferentially in certain brain compartments. Brain regions, and other tissues from 19 East Greenland polar bears (13 males, 6 females) were analyzed for 4 PFSAs (C4, C6, C8, C10), 10 PFCAs (C6 – C15), and 13 precursor compounds. Brain samples were taken from eight different compartments: pons and medulla, cerebellum, frontal cortex, occipital cortex, temporal cortex, striatum, thalamus and hypothalamus. Some highlights of our findings showed that in the brain, PFOS was present at the highest levels (35.2 ng/g ww), followed by perfluorotridecanoic acid, PFTrIA, (31.4

ng/g ww). Long-chain PFCAs (C10-C15) and PFOS were all significantly ( $p < 0.05$ ) positively correlated with lipid content. Significant differences ( $p < 0.05$ ) between compartments for long-chain PFCAs (C11-C14) were present, and positively correlated with extractable lipid content. Positive age correlations were found for PFSAs (C6, C8, C10), long-chain PFCAs (C10-C15), and FOSA. Until now, it has been believed that relative to protein-associated interactions, lipids play a more minor role in the distribution of PFAAs in the body; however, these results suggest that long-chain PFCA distribution is indeed influenced by lipid content in the brain.

**TP053 Developmental Toxicity of Perfluorinated Phosphonic Acids in Mice** K. Tatum-Gibbs, UNC-EPA, Post-doc; K. Das, B. Grey, C. Wolf, A. Watkins, B. Abbott, USEPA, Toxicity Assessment Division; M. Strynar, A. Lindstrom, USEPA, Human Exposure and Atmospheric Sciences Division; C. Lau, USEPA, Toxicity Assessment Division. Perfluorinated phosphonic acids (PFPA) are a third member of the perfluoroalkyl acid (PFAA) family, and are structurally similar to the perfluoroalkyl sulfonates and perfluoroalkyl carboxylates. PFPA are used primarily as a surfactant defoaming agent in pesticide production. Recently, these emerging chemicals have been detected in the environment, particularly in surface water as well as in effluent of wastewater treatment plants at concentrations ranging from pg/L to the low ng/L range. The presence of these chemicals in wastewater suggests human exposure; currently there is limited toxicological data concerning the potential health risks associated with exposure to this class of PFAAs. Previous studies from our laboratory have identified developmental toxicity associated with gestational exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). The current study examines the potential adverse developmental effects of PFPA in the mouse. A mixture of PFPA (Masurf-780) was given to timed-pregnant CD-1 mice by oral gavage daily on gestation days (GD) 1-17 at doses of 5, 10, 20, 30 or 40 mg/kg; controls received deionized water vehicle. At GD 17 (24 hours after the last treatment), mice from each dose group were subdivided and approximately half were sacrificed via decapitation for maternal and fetal examinations. PFPA did not alter maternal weight gains, but significantly increased maternal liver weights at term (GD-17) in a dose-dependent manner. PFPA did not influence the number of live fetuses or fetus weight observed at GD-17, except in the 40 mg/kg group where some mortality was observed. In contrast, fetal liver weights were significantly increased at doses greater than 5 mg/kg. Neonatal survival and growth were monitored through postnatal day 42 where significant changes were only seen in the highest dose group; whereas increased liver weight persisted in all dose groups. These results thus suggest that PFPA exposure during pregnancy did not compromise neonatal survival and postnatal growth to the extent seen with PFOS and PFOA, but the hepatic effects appeared to be common to all classes PFAAs. This abstract does not necessarily reflect USEPA policy.

**TP054 Perfluorinated Phosphinates and Polyfluoroalkyl Phosphoric Acid Diesters in Sediments from the Great Lakes Region** R. Guo, Univ of Toronto; E. Reiner, Ontario Ministry of the Environment, Univ of Toronto, Dept of Chemistry; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; C. Marvin, Environment Canada; S. Mabury, Univ of Toronto; S. Bhavsar, Ontario Ministry of the Environment; G. Tomy, Fisheries and Oceans Canada; M. Simcik, Univ of Minnesota. Perfluorocarboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) have been the focus of environmental research and monitoring studies of perfluorinated chemicals (PFCs) over the past decade. More recently, other PFCs have attracted attention, including the polyfluoroalkyl phosphoric acid diesters (diPAPs) and perfluorinated phosphinates (PFPIs). Following method improvements to enhance extraction efficiencies, we examined sediments from Lake Ontario, one of the Laurentian Great Lakes, and Lake Simcoe in the more populated region of south-central Ontario, and sediments from remote lakes in or near the Great Lakes region, for the occurrence and deposition trends of diPAPs and PFPIs. In the Lake Ontario and the Lake Simcoe cores, PFCAs and PFSAs, also included in the study, increased from down core to peak concentrations in surface sediments. PFOS was the dominant PFC (1-12 ng/g in Lake Ontario sediment core, 0.1-1.9 ng/g in Lake Simcoe). For PFPIs, 6:6PFPI was dominant in Lake Ontario and Lake Simcoe sediment cores with concentrations ranging from 0.03 to 0.5 and 0.3 to 1.3 ng/g, respectively. PFPI concentrations are higher in sediment from Lake Simcoe than those from the Lake Ontario sediment core, contrary to the PFCAs and PFSAs. The PFPIs increased in both cores from the 1960s to recent slices. For the diPAPs, 6:2 diPAP was dominant in



both sediment cores, with concentrations ranging from 0.03 to 0.64 ng/g in the Lake Ontario core and 0.05 to 9.1 ng/g in the Lake Simcoe core. Similar to PFPIs, concentrations of the diPAPs generally increase from down core peaking in recent or surface sediments. Sediment concentration trends and fluxes will be compared to those collected from more remote lakes in the region assess if these PFC compounds are subject to atmospheric transport processes.

**TP055 6:2 FTOH Aerobic Biodegradation in Freshwater Sediment and Activated Sludge** L. Zhao, Nankai Univ, MOE Key Laboratory of Pollution Processes and Environmental Criteria; P.W. Folsom, P.K. Cooper, B.W. Wolstenholme, E.I. du Pont de Nemours & Company, Inc; H. Sun, Nankai Univ, MOE Key Laboratory of Pollution Processes and Environmental Criteria; N. Wang, R.C. Buck, E.I. du Pont de Nemours & Company, Inc. The widespread detection of anthropogenic perfluoroalkyl sulfonates [PFASs,  $F(CF_2)_nSO_3^-$ ,  $n = 4 - 8$ ] and perfluorocarboxylates [PFCAs,  $F(CF_2)_mCOO^-$ ,  $m = 4 - 12$ ] in the environment has lead to investigations about their sources, fate, transport, and behaviors in different environmental matrices. Products containing long-chain PFASs and PFCAs or potential precursors are being phased out and replaced with shorter-chain alternatives based on perfluorobutane sulfonate and carboxylate, 6:2 fluorotelomer alcohol [6-2 FTOH,  $F(CF_2)_6CH_2CH_2OH$ ], and fluorinated ethers. Currently, limited information is available on the environmental fate and behaviors of these alternatives. With increased production of 6:2 FTOH-based materials, it is important to understand the fate and behaviors of 6-2 FTOH in relevant environmental matrices. The purpose of this study was to investigate 6:2 FTOH degradation kinetics, identify potential transformation products, and elucidate biotransformation pathways in freshwater sediment and activated sludge. The 6:2 FTOH and  $^{14}C$ -labeled [1,2- $^{14}C$ ] 6:2 FTOH were dosed into the "semi-static system" of sediment or activated sludge to determine 6:2 FTOH partitioning behavior and biodegradation potential. The experimental details such as setup, sample processing, analytical methods will be discussed. The results indicate that 6:2 FTOH can be rapidly transformed (T<sub>1/2</sub> 28 days) with 5:3 acid and volatile intermediates such as 5:2 ketone [ $F(CF_2)_5C(O)CH_3$ ] and 5:2 sFTOH [ $F(CF_2)_5CH(OH)CH_3$ ] as the major transformation products at day 28. Proposed 6:2 FTOH biotransformation pathways in activated sludge and aerobic sediment systems will also be presented.

**TP056 Challenges Associated with the Analysis of Fluorotelomer Sulfonates in Environmental Samples** J.P. Benskin, AXYS Analytical Services Ltd; M.B. Woudneh, AXYS Analytical Services Ltd, R&D Chemist; J. Pape, Axys Analytical Services Ltd.; M. Ikonomou, Fisheries and Oceans Canada, Contaminant Sciences Section; R. Grace, Axys Analytical Services; J.R. Cosgrove, AXYS Analytical Services Ltd. In contrast to perfluorinated sulfonates (PFHxS, PFOS, etc.), which are widely reported in humans and wildlife, there are comparatively little data on the environmental occurrence of their polyfluorinated counterparts, the fluorotelomer sulfonates (FTSs). The few data available indicate the latter compounds can be major contaminants of groundwater near airports (up to tens of mg/L); consequently, there is an urgent need for analytical methods capable of accurately quantifying the distribution of FTSs in environmental samples. Here we report a new method for determination of 4:2, 6:2, and 8:2 FTSs in water and biosolid matrices. Despite the apparent structural similarities between FTSs and perfluorinated sulfonates, analysis of the former by 'traditional' methods (i.e., methods designed for PFOS and PFHxS) resulted in significant matrix enhancement (i.e., >200%) of FTS congeners. Attempts at reducing this enhancement by dilution or clean-up with activated carbon were largely unsuccessful; as a result, a stand-alone method for the extraction and analysis of FTSs was developed. Absence of matrix effects in real samples analyzed by this new method was confirmed by standard addition, and detection limits for each congener were in the low ng/L range for a 500mL water sample. These data highlight the need for isotope-labelled internal standards for fluorotelomer sulfonates, and the significant differences in ionization behavior which exist between perfluorinated and polyfluorinated sulfonates.

**TP057 Perfluoroalkyl and Polyfluoroalkyl Substances (PFASs) in the Environment: A Review of Terminology and Hierarchy** J. Conder, ENVIRON International Corporation; R. Buck, E. I. duPont de Nemours & Co., Inc., Chemicals & Fluoroproducts, DuPont, Chemicals & Fluoroproducts; J. Franklin, CLF-Chem Consulting; U. Berger, Stockholm Univ, Dept of Applied Environmental Science (ITM); I. Cousins, Stockholm

Univ; P. de Voogt, Univ of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics; A.A. Jensen, Nordic Institute of Product Sustainability, Environmental Chemistry and Toxicology; K. Kannan, New York State Dept of Health, Wadsworth Center; S.A. Mabury, Univ of Toronto, Dept of Chemistry; S.v. Leeuwen, Institute for Environmental Studies, VU Univ. Perfluoroalkyl and polyfluoroalkyl substances (PFASs), including the perfluoroalkyl acids (PFAAs) perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been detected in the environment, wildlife, and humans. Owing to the variety and complexity of PFASs, the terminology and acronyms presented in the historic scientific literature for these substances are inconsistent. This paper will present a condensed overview of descriptive terminology, names and acronyms, and organizational hierarchy for PFASs to promote a sound, unified lexicon for use by the global scientific community that is clear, specific and descriptive for individual substances and groups of substances. We will illustrate the nuances and recommended uses of the terms "perfluoroalkyl", "polyfluoroalkyl", "fluoropolymers", "perfluorinated", "fluorinated", "polyfluorinated", and other phraseologies as well as the acronym PFC for perfluorocarbons. We propose that environmental PFASs of interest are best understood by segregation into general families: 1) non-polymer perfluoroalkyl substances (e.g., PFAAs which include PFOS and PFOA); 2) non-polymer polyfluoroalkyl substances (e.g., fluorotelomer alcohols, FTOHs); and 3) fluorinated polymers. We describe recommended terminology for classes of substances as well as individual substances. This hierarchical structure not only provides a better understanding of chemical structure, similarities, differences and nomenclature, but also facilitates a better grasp of uses and origins (e.g., electrochemical fluorination versus fluorotelomer process).

**TP058 Phosphorus Containing Organofluorine Surfactants in Indoor Residential Dust** A.O. De Silva, Environment Canada, Water Science & Technology Directorate, Environment Canada, Water Science and Technology; C. Allard, Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate, Environment Canada, Canada; G. Webster, School of Population and Public Health, Univ of British Columbia, Canada. Indoor dust is an important route of human exposure to many persistent organic pollutants (POPs), including perfluorinated compounds (PFCs). Anionic emerging PFCs are of particular interest including polyfluorinated phosphoric diesters (diPAPs), perfluorophosphonates (PFPA) and perfluorophosphinic acids (PFPIs). diPAPs are used in paper treatment products and were measured in wastewater sludge, human sera, and are metabolized to PFCAs in rodents. PFPA and PFPIs are used as leveling and wetting agents in waxes and coatings, defoaming agents in pesticide formulations and are environmentally persistent. PFPA and PFPIs were measured in surface waters from Canada and PFPIs were observed in human serum. To date, none of these compounds have been monitored in indoor dust in Canada or elsewhere. This study describes the development of analytical methodology to measure emerging PFCs in 100 indoor dust samples collected in 2007-08 from the homes of pregnant participants enrolled in the Vancouver-based Chemicals Health and Pregnancy study (CHiRP). The method consists of solvent extraction followed by carbon SPE clean-up and analysis by LC-MS/MS. Each dust sample was analyzed in duplicate using 0.1 g. The method was evaluated for accuracy via spike and recovery, matrix effects, and precision (n=4). Significant matrix effects were noted for 6:2/6:2 diPAP (50% matrix suppression) and C6 PFPA (150% matrix enhancement). C6 PFPA concentrations were internally corrected for matrix effects using a surrogate with a Cl substituent (Wellington Labs, Guelph, ON). C13-labeled  $F(CF_2)CO_2-$  or  $F(CF_2)SO_3-$  surrogates were used as a recovery standard for diPAP, PFPA, and PFPI analytes. Extraction recovery corresponded to 64% 6:2/6:2 diPAP, 88% 10:2/10:2 diPAP, 109% C6 PFHxPA, 42-47% PFOPA, 74% C6/C6 PFPI, 84% C6/C8 PFPI, and 89% C8/C8 PFPI. Precision was based on RSD of replicate (n=4) analysis of 2 samples. %RSD was < 10 for all analytes except for C6/C6 PFPI (RSD < 25%). Preliminary analysis indicated that the predominant PFC was 8:2/8:2 diPAP ranging from 2000 to 60,000 ng/g. Other diPAP congeners were observed in every sample. PFPA were >LOD in < 50% of samples and ranged from 3 to 200 ng/g, mainly C6 PFPA. Both the C6/C6 (0.1 to 37 ng/g) and C6/C8 PFPI (0.5 to 52 ng/g) were measured in every sample. Widespread detection diPAPs and PFPIs indicate ubiquitous presence in indoor residential dust and potentially a significant source of exposure.

**TP059 Use of Microarray Analyses to Characterize Impacts of Hyper-saline Conditions on Pesticide Toxicity in Salmonids** L.A. Maryoung,



Univ of California – Riverside, Environmental Science, Univ of California – Riverside; R. Lavado, Univ of California, Riverside, Environmental Science; E.P. Gallagher, P.L. Stapleton, T.K. Bammler, R. Beyer, F. Farin, Univ of Washington, Environmental and Occupational Health Sciences; D. Schlenk, Univ of California, Riverside, Environmental Science. Acclimation to hypersaline environments has been shown in previous studies to enhance the acute toxicity of thioether organophosphate and carbamate pesticides to salmonids. While acute toxicity is likely due to enhanced formation of anti-cholinesterase metabolites, chronic impacts are less clear. In the current study, juvenile coho salmon (*Oncorhynchus kisutch*) were exposed to varying levels of salinity (< 0.5‰, 8‰, 16‰, and 32‰) for one week and mRNA was collected from the livers, gills, and olfactory rosettes. Microarray hybridization was used to determine differences in gene expression in the various tissues for the different treatments. Using a two fold cutoff, it was found that 20 genes were differentially expressed in livers between all treatments, 26 in gills, and 51 in olfactory rosettes. Further analysis of the data is ongoing, with the next steps including GO analysis and target clustering, to better understand the mechanisms of how these pathways are impacted by pesticides and hypersaline acclimation. (NIEHS P30ES07033).

**TP060 Exploring Impacts of Climate-induced Changes in Temperature and Salinity on Freshwater Gastropods** J. Suski, Texas Tech Univ, Dept of Biological Sciences, , United States Environmental Protection Agency, Risk Assessment Division; C.J. Salice, Texas Tech Univ/TIEHH, Environmental Toxicology, Assistant Professor, Texas Tech Univ. Climate change predictions for some regions indicate more extreme climate fluctuations including changes in precipitation patterns and global temperature changes. Freshwater systems will be impacted by predicted climate changes and those that are dependent on rainfall for filling or are shallow may be particularly vulnerable. Although precipitation volume may not change, patterns in rainfall may severely limit available habitat as misting rain leaves little accumulation in ponds and extreme downpours can result in lower overall water retention rates. This is especially significant in arid or semi-arid regions where many freshwater systems are dependent on filling of ponds. Playas are small ephemeral wetlands in the Southern High Plains and provide critical habitat for a wide variety of terrestrial and aquatic organisms. Climate change predictions for the Southern High Plains in conjunction with high water demand will lead to increases in salinity and temperature within freshwater ponds and playa wetlands. Locally, gastropods comprise a large portion of the invertebrate biomass and are important prey species for many aquatic and terrestrial animals. Hence, understanding how temperature and salinity impact gastropods may provide insight into higher-level wetland effects. We exposed juveniles of field collected ramshorn snails (*Planorbella tenuis*) to a range of environmentally relevant salinities (250 – 4000  $\mu\text{S cm}^{-1}$ ) and temperatures (21 – 32°C) as these two stressors can and will co-occur. Exposure duration was 14-days. Results showed that somatic growth rates of juvenile snails were positively correlated with temperature regardless of salt stress. Additionally, there were no adverse effects of salt stress. However, there was a slight increase in mortality at higher temperatures. The lack of a deleterious effect of salt stress is in contrast to previous studies in which we demonstrated negative effects to salt exposures that were much longer in duration. Collectively, our data suggest that negative effects of salt may take a relatively long time to manifest and as yet, do not appear to be exacerbated by the range of temperatures we used here. Future efforts will extend the study duration to include fecundity and also potential impacts of increased salt and temperature to predator avoidance behaviors.

**TP061 Benzotriazole UV Stabilizers in the Environment** H. Nakata, Kumamoto Univ, Graduate School of Science and Technology, Kumamoto Univ, Dept of Environmental Science; S. Murata, R. Shinohara, H. Yanagimoto, N. Shikata, Kumamoto Univ; M. Watanabe, National Institute for Environmental Studies; T. Isobe, S. Tanabe, Ehime Univ; T. Kunisue, K. Kannan, Wadsworth Center, SUNY at Albany. Benzotriazole UV stabilizers (BUVSs) have been used for various industrial and consumer products. Recently, the occurrence and contamination of BUVSs have reported in the aquatic ecosystems, but little information is available on the source, pathway, and distribution of BUVSs in the environment. In this study, we investigated the concentrations, bioaccumulation, spatial distribution, temporal trends and source identification of BUVSs in the environment. BUVSs were detected in marine and terrestrial organisms, such as mussels, fish, mammals, birds, and human, which suggest a bioaccumulation of BUVSs through the food-chain. The bioconcentration factor (BCF) of UV-327

(CAS #: 3864-99-1) in marine mammals was 33,300, which was similar to that of legacy POPs, HCHs (hexachlorocyclohexane). BUVSs were detected in oysters and mussels collected from nine Asian countries (Cambodia, China, India, Indonesia, Japan, Korea, Malaysia, Philippines, Vietnam) and the US, indicating a widespread contamination of BUVSs in the Asia-Pacific regions. Sediment core samples collected from Tokyo Bay were analyzed BUVSs. UV-326, UV-327, and UV-328 were detected, and the levels increased from the depth 5-10 cm to the top layers. Sediment dating data suggested that BUVSs has emerged in first of the 1970s in Japan, and the contamination has been continued during past 30 years. To determine the sources of BUVSs in the environment, influent, effluent and sludge samples collected from WWTPs and road dust were analyzed. BUVSs were detected in all samples analyzed, and UV-326 was the dominant compound in wastewater samples, followed by UV-328, and UV-327. This indicates the current usage of BUVSs in cosmetics and household materials. In road dust, significant correlations were obtained between BUVSs concentrations and traffic density. These results indicate that WWTPs and road dust may be a potential source of BUVSs in the aquatic ecosystems.

**TP062 Does Anti-inflammatory Drugs Bioconcentrate in Wild Fish Bile?** J. Brozinski, Abo Akademi Univ, Laboratory of Organic chemistry, ?bo Akademi Univ, Laboratory of Organic chemistry; M. Lahti, A. Oikari, Univ of Jyväskylä, Dept of Biological and Environmental Sciences; L. Kronberg, Abo Akademi Univ, Laboratory of Organic chemistry. The usage of pharmaceuticals is increasing worldwide. For example, the anti-inflammatory drugs diclofenac (DCF), naproxen (NPX) and ibuprofen (IBF) has been found as micropollutants in river water downstream of the discharge point of wastewater treatment plants. We have exposed rainbow trout (*Oncorhynchus mykiss*) to DCF, NPX and IBF in aquaria at near environmental concentrations, and have determined the up-take and metabolism of the drugs by studying bile samples. The bile samples were analyzed with LC-MS/MS methods. The identification of the formed metabolites were based on the exact mass determinations by a time-of-flight mass analyzer (Q-TOF-MS) and on the studies of fragments and fragmentation patterns of the precursor ions by ion trap mass analyzer (IT-MS). The quantitative analyses were performed by triple quadrupole mass analyzer (QqQ-MS). The studied compounds and their metabolites, mainly acyl glucuronides, were found in the fish bile. Also it was evident that the fish bioaccumulated the compounds in the bile. The bioaccumulation factors were 500, 1200, and 61 000 for DCF, NPX and IBF, respectively. Currently, we are collecting bile samples from wild fish from a river/lake system, which is heavily loaded with treated municipal wastewater. The objective of the work is to find out whether also wild fish bioaccumulate and metabolize pharmaceuticals. Results of this study will be presented.

**TP063 Levels of Legacy POPs and Emerging Contaminants in the Tonlé Sap Watershed, Central Cambodia** S. Bayen, National Univ of Singapore, Dept of Civil and Environmental Engineering; H. Zhang, National Univ of Singapore, Dept of Civil and Environmental Engineering, National Univ of Singapore; B. Tan, DSO National Laboratories; N. Steenkeste, FONDATION MERIEUX; M. Chou, Univ of Health Science, Faculty of Pharmacy; P. Lim, Ministry of Water Resources and Meteorology; B.C. Kelly, National Univ of Singapore, Dept of Civil and Environmental Engineering. Tonlé Sap lake, in central Cambodia, is the largest natural lake in Southeast Asia (~18,000 km<sup>2</sup> during southwest monsoon maxima). The lake supports a vibrant fishery which provides a large proportion of the nutritional requirement of the human population of the country. The objective of the present study was to investigate the occurrence and levels of organic contaminants in water, sediments and biota from this important watershed. Samples, including surface water (n= 18), sediments (n= 18), bivalves (n = 5 pools) and fish (n = 257, across several trophic levels, both muscle and liver), were collected in March 2011 at four locations on the lake. Filtered water samples were extracted using SPE. Accelerated Solvent Extraction (ASE) and sonication were used to extract filters, sediments and tissue samples. After cleanup, extracts were analyzed by gas chromatography – tandem mass spectrometry (GC-MS/MS). Target analytes included legacy persistent organic pollutants (POPs) but also emerging contaminants of concern, including several brominated flame retardants (BFRs) and synthetic musks. <sup>13</sup>C mass-labeled compounds were used for quantification (isotope dilution). QA/QC measures included procedural blank analyses, recovery tests and replicate analyses. Observed concentrations of the various contaminants in field collected samples were compiled and compared with levels from other locations in

Southeast Asia and elsewhere. The partitioning behaviour and distribution of the hydrophobic chemicals between particulate and dissolved fractions is presented and discussed. Eventually, the concentrations of contaminants in fish from different trophic levels will be compared to highlight the biomagnification mechanisms. This study provides the first comprehensive dataset for legacy POPs and emerging contaminants in this important Cambodian watershed.

**TP064 Bioavailability and Uptake of Hexabromocyclododecane in an Aquatic Food Chain** A.K. Karjalainen, Univ of Jyväskylä, Dept of Biological and Environmental Science; S. Huhtala, Finnish Environment Institute; J. Karjalainen, T. Keskinen, Univ of Jyväskylä; J.V. Kukkonen, M. Leppanen, K. Maenpaa, Univ of Eastern Finland; P. Sainio, M. Verta, Finnish Environment Institute. Hexabromocyclododecane (HBCD), a brominated aliphatic cyclic hydrocarbon, is currently a third most used additive brominated flame retardant (BFR) following tetrabromobisphenol A and polybrominated diphenylethers (PBDEs). The synthesized technical HBCD mixture contains mainly three stereoisomers termed  $\gamma$ -,  $\beta$ - and  $\alpha$ -HBCD with the  $\gamma$  isomer being the predominant component. Technical HBCD enters the environment through emission during its production or flame retarded products and by leaching from consumer products. As a result of its physical and chemical properties and widespread use, it is now a ubiquitous contaminant in the environment and humans. Contrary to PBDEs, increase in environmental concentrations has not levelled off but seem to be increasing. HBCD has a high bioaccumulation potential and may disrupt endocrine function both in animals and humans. Bioisomerisation in aquatic food chain can occur leading to altered proportion of stereoisomers in animals compared to the technical product or the primary source for uptake such as sediment. The aim of this study was to investigate the bioavailability, trophic transfer and bioisomerisation of HBCD in a simplified aquatic food chain model. Bulk of Oligochaete worm *Lumbricus variegatus* was firstly exposed to HBCD-spiked sediment at environmentally realistic concentration to yield information on the bioavailability potential of HBCD in sediment. Additionally sublethal endpoints and bioisomerisation were measured. Secondly, we measured the partition ratio of HBCD at steady state between artificial freshwater and polydimethylsiloxane (PDMS) fibre, and used it to plan appropriate exposure conditions for passive dosing of masses of *Chironomus* sp. 4th instar larvae with saturated PDMS silicone. The contaminated larvae were used as a relevant food source for ruffe (*Gymnocephalus cernuus*) at environmentally realistic concentrations. Subsequently, uptake kinetics, trophic transfer and bioisomerisation fate of HBCD in the simplified food chain model were measured. Liquid scintillation counting (LSC) and ultra performance liquid chromatography tandem mass spectrometry (UPLC-TQMS) was used for radiolabelled and non-labelled HBCD, respectively. This study yields new information for risk assessment of brominated flame retardants.

**TP065 The Influence of Surface Charge on Gold Nanosphere Accumulation in a Simple Aquatic Food Chain** A. Wray, Clemson Univ, Institute of Environmental Toxicology; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology (ENTOX). Over the last two decades, nanotechnology has ascended to industrial prominence; a fact attributed to the seemingly limitless potential for enhancement, innovation and invention. With increasing ubiquity in the market the release of NPs into the environment, whether intentional or accidental, becomes inevitable. The goal of our research was to characterize the influence of particle properties on the uptake and distribution of gold nanospheres in a simulated aquatic food chain. Expanding on our previous investigation of size dependent uptake and elimination, this study focused on gold nanoparticles exhibiting different surface chemistries (anionic, cationic, and neutral) to shed light on the mechanism(s) through which these particles accumulate in *Selenastrum capricornutum* and *Daphnia magna*. In mammalian studies, both in vitro and in vivo, surface charge has been identified as a key component controlling GIT absorption of NPs thus we expected it to play a similar role in our aquatic organisms. Additionally, our gold nanoparticle stocks were incubated with natural organic matter, to examine a more environmentally relevant scenario of nanoparticle exposures to our model organisms. In all scenarios, the uptake and elimination rate constants were derived to predict how surface chemistry influences accumulation of particles and how the presence of biotic ligands can enhance or devalue these influences.

**TP066 Bioaccumulation Modeling of Perfluorinated Compounds in an Aquatic Food Web** J.P. Benskin, AXYS Analytical Services Ltd; M. Ikonomou, Fisheries and Oceans Canada, Contaminant Sciences Section; F.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment), Simon Fraser Univ; M.B. Woudneh, AXYS Analytical Services Ltd, R&D Chemist; J.R. Cosgrove, AXYS Analytical Services Ltd. Among the many differences between perfluorinated compounds (PFCs) and legacy persistent organic pollutants (POPs) is the tendency for PFCs to accumulate in protein rich tissues, as opposed to lipids. Predicting the bioaccumulation of PFCs using models designed for legacy POPs is difficult, since these methods rely on lipid partition coefficients (e.g., Log Kow), which are difficult to measure for PFCs, and largely inappropriate for predicting PFC behavior in wildlife. New approaches which account for the "proteinophilic" nature of PFCs are necessary to accurately predict their disposition in biota. Here we report a comprehensive examination of PFCs in a marine food web from False Creek (FC) and Burrard Inlet (Vancouver, BC, Canada) and application of these data in developing a mass-balance bioaccumulation model for PFCs. Representative samples of sediment, sea water, and biota (algae, bivalves, fish, etc.) were collected and analyzed for a suite of PFCs, including perfluoroalkyl carboxylates, sulfonates, sulfonamides, sulfonamido acids, telomer sulfonates, and telomer acids, and isomer profiles. In water, the highest total PFC concentrations (tens of ng/L) were observed in the East Basin (FC), where PFOS was the dominant congener. PFC concentrations decreased rapidly moving into the Central/ North Harbour (FC) and Burrard Inlet, where perfluorooctanoate (PFOA) and perfluorohexanoate (PFHxA) were the major PFCs. Isomer profiles of PFOA were exclusively linear, indicating contributions predominantly from a telomer manufacturing source. The utilization of protein partition coefficients and field data for model development is discussed.

**TP067 Perfluorochemicals in Soil and Produce from Home Gardens** C. Huset, K. Souther, MN Dept of Health, Organic Chemistry Unit. Ground and drinking water in Washington County, Minnesota have been monitored for perfluorochemicals (PFCs) since 2004. Several waste disposal sites located in Washington County were used by 3M to dispose of manufacturing wastes in the 1950s-1970s were determined to be the source for these PFCs in the ground and drinking water. While exposure to PFCs through drinking water has been reduced by changes in water source, or drinking water treatment, there are other exposure routes related to the contaminated drinking water that may still affect residents in these contaminated areas. There is potential for produce grown in gardens from these contaminated areas to accumulate PFCs through irrigation with contaminated water. There is also potential for uptake from soil with historic PFC contamination (arising from historical irrigation with PFC-containing water). The goals for this study are to determine the extent to which the garden soil is contaminated with PFCs and to determine the uptake of PFCs into fruits and vegetables grown on these sites. Twenty home gardens in the communities with PFC contamination as well as four control sites (sites located outside the contaminated area, with no known PFC contamination) were sampled for soil as well as produce collected throughout the growing season. 278 produce samples were collected, including 43 tomatoes, 23 peppers, 16 cucumber, 16 squash, 16 beans, 16 peas as well as onions, carrots, corn, berries and herbs. The PFCs of interest, PFBA, PFPeA, PFHxA, PFOA, PFBS, PFHxS, PFOS were analyzed in soil and produce using LC/MS/MS after homogenization and extraction. Quantitation limits, which vary depending on analyte and produce type, range from 0.05 ng/g to 0.15 ng/g. Data presented will compare soil, produce and water concentrations from the various sites.

**TP068 Analysis of Bisphenol A in a Leafy Vegetable Using an Isotope Dilution Technique, Liquid-Liquid Extraction, and GC-Tandem MS Analysis** J. Lu, J. Wu, Univ of Florida/IFAS-IRREC, Soil and Water Science; P. Stoffella, Univ of Florida/IFAS-IRREC, Horticulture; P. Wilson, Univ of Florida, IFAS, IRREC- Soil & Water Science. An isotope dilution method was developed for determination of bisphenol A (BPA) in leafy vegetables by gas chromatography with tandem mass spectrometry (MS/MS). The internal standard (IS) C13 labeled BPA (ring-13C12) and the surrogate 17 $\alpha$ -ethinylestradiol (17 $\alpha$ -EE2) were added at the beginning of the extraction. The matrix was homogenized and extracted ultrasonically using acetone. Effective elimination of impurities from the extract was obtained with a clean-up procedure involving liquid-liquid extraction by acetonitrile (ACN) saturated hexane to remove most of the chlorophyll, which can lead to the failure of subsequent derivation. The ACN extract was

subject to an unspecific acid hydrolysis procedure to release the conjugated BPA as free BPA. The silylating derivatization process was performed with trimethylchlorosilane (TMCS) and pyridine. Multiple reaction monitoring (MRM) mode was performed since BPA and the IS co-eluted at 13.3min. The precursor ions for BPA and internal standard were  $m/z$  357 and  $m/z$  369, respectively. The excitation amplitude voltage for BPA was 0.7 V while that for the IS was 0.8 V. Instrument limit of detection (LOD) and limit of quantification (LOQ) were 1.8  $\mu\text{g/L}$  and 6.0  $\mu\text{g/L}$ , respectively, based on 1  $\mu\text{L}$  injections of 1 mg/L BPA standard solution. The BPA concentrations in lettuce samples ranged from 6.6  $\mu\text{g/kg}$  fresh weight to 150.0  $\mu\text{g/kg}$  fresh weight. Hexane clean-up is an essential step for this analysis method. The results illustrate a useful, alternative method for analysis of BPA content in leafy vegetables.

**TP069 Uptake and Biotransformation of 17 $\beta$ -trenbolone by the Common Bean Plant (*Phaseolus vulgaris*)** B.R. Blackwell, Texas Tech Univ/TIEHH, Environmental Toxicology, Texas Tech Univ, Environmental Toxicology; A. Karnjanapiboonwong, Texas Tech Univ/TIEHH, Environmental Toxicology, Missouri Univ of Science and Technology, Civil, Architectural and Environmental Engineering; T. Anderson, P.N. Smith, Texas Tech Univ/TIEHH, Environmental Toxicology. Manure from livestock feeding operations is commonly applied to agricultural fields as an alternative to chemical fertilizers. Trenbolone acetate is an anabolic growth promoter frequently utilized in beef cattle feeding operations. Metabolites of trenbolone acetate can be present in manure and subsequently applied to fields, however, the fate of trenbolone after application to agricultural fields is not fully understood. The objective of this study was to investigate uptake and biotransformation of the trenbolone acetate metabolite 17 $\beta$ -trenbolone (17 $\beta$ -TbOH) using the common bean plant (*Phaseolus vulgaris*). Vegetated ( $n=20$ ) and control sands ( $n=16$ ) were amended with 17 $\beta$ -TbOH at a level of 1  $\mu\text{g/g}$  of sand once per week for a total of 4 weeks. Plants and controls were collected each week and separated into sand, above-ground plant portion, below-ground plant portion, and analyzed for 17 $\beta$ -TbOH, 17 $\alpha$ -trenbolone, and trenbolone (TbO). Low levels of 17 $\beta$ -TbOH were taken up into the roots of plants and, to a much lesser extent, transported throughout the plant. Extensive transformation to the metabolite TbO occurred in plants and in vegetated sands while minimal TbO was detected in control sands. After four weeks, maximum concentrations of total trenbolone in fresh plant tissues were 38.5  $\mu\text{g/g}$  in roots and 0.290  $\mu\text{g/g}$  in leaves. These results demonstrate 17 $\beta$ -TbOH can be taken up into plants and transformed to various trenbolone metabolites.

**TP070 Pharmaceuticals in Sewage Sludge Compost and Their Uptake from Fertilized Soil by Food Plants** L. Nei, Tallinn Univ of Technology Tartu College, Dept of Environmental Protection; M. Lillenberg, Estonian Univ of Life Sciences; E. Haiba, Tallinn Univ of Technology, Tartu College; K. Kipper, K. Herodes, Tartu Univ, Institute of Chemistry. The potentially hazardous organic compounds that may be present in sewage and sewage sludge number in the thousands. Fortunately, the concentrations of toxic organic chemicals are usually low and for most of them their plant bioaccumulation factors are small. Still, compounds with strong sorption and recalcitrant to degradation remain in surface soils and have the potential to subsequently be taken up by plants. However, very limited information is currently available. Previous research has focused primarily on plant uptake of veterinary pharmaceuticals that are associated with animal waste, that is, manures, and demonstrated their potential to accumulate in plants. Humans may be exposed to residues of drugs in the environment by a number of routes including the consumption of crops that have accumulated substances from fertilized soils. As the compost made from sewage sludge contains detectable amounts of pharmaceutical residues, there was a need to determine the significance of uptake into plants from soil under "real" conditions as a potential migration route for pharmaceuticals in the environment. Therefore, the current study was conducted to determine the potential for tetracyclines, fluoroquinolones and sulfonamides to be taken up by food plants from soil fertilized with sewage sludge or its compost. The results of this work clearly show that pharmaceuticals are able to accumulate in plants. This phenomenon remarkably depends on the nature and concentration of pharmaceuticals and the soil type. Before using the sewage sludge compost as a fertilizer, it should be carefully tested against the content of different pharmaceuticals. The content of pharmaceuticals in the compost made from sewage sludge may easily lead to the elevated concentrations in food plants, if the compost is used as a fertilizer. This work should be continued by the development of novel and more efficient sewage sludge treatment

technologies, leading to intelligent solutions of environmental problems related to sewage sludge exploitation.

**TP071 Effects of Feeding Rate and Loading Density on Bioaccumulation of PCBs in Oligochaete *Lumbricus variegatus*** N. Billa, Environmental Protection Agency; T. Lahren, US Environmental Protection Agency, Mid-Continent Ecology Division; T.L. Highland, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; J.R. Hockett, US Environmental Protection Agency, ORD; D. Hoff, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; D.R. Mount, US Environmental Protection Agency, ORD; T.J. Norberg-King, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; L.P. Burkhard, USEPA, Mid-Continent Ecology Division. Sediment tests with aquatic organisms can provide valuable information about potential toxicity and the bioavailability of polychlorinated biphenyls (PCBs) to the organisms. The US-Environmental Protection Agency 28-day *Lumbricus variegatus* bioaccumulation test for sediments when successfully performed often has poor growth of the *L. variegatus*. Potential causes of poor growth include poor nutritional quality of the sediment, sublethal toxicity effects, and/or too much competition for the available food in the sediment (i.e., too many organisms for the available food). The test, as currently configured, has conditions of no feeding and a loading rate of organisms in the test chambers of no less than a ratio of 50:1 of organic carbon to organism dry weight (USEPA, Test Method 100.3). The objectives here are to investigate the effects of loading density and feeding on the growth of the *L. variegatus* and their subsequent impacts on the bioaccumulation of PCBs by the organisms. A preliminary test was performed with 2 loading densities, 100:1 and 25:1 (ratio of organic carbon to organism dry weight) and 3 feeding rates (0, 2, 4 times feeding per week) using a Hudson River (HR) sediment contaminated with PCBs at 2.6 ppm along with our standard control sediment (West Bearskin). Results of the preliminary study demonstrated that PCBs residues were influenced by feeding and loading rates. Growth of the organisms increased with increase feeding rate suggesting more available nutrition from external food provided. A significant difference (Tukey honestly significant difference,  $\alpha = 5\%$ ) in the growth of the organisms was observed with low loading (100:1). No significant difference in the growth was observed with high loading (25:1). These results suggest limited food availability per organism in high loading treatments. PCB residues declined with increasing feeding rate and residues increased with increased loading. Growth of the organisms with feeding in combination with loading rates was inversely related to the bioaccumulation of PCB residues. Following this study, we performed another 28-day exposure with no feeding and four different loading densities based on the organic content of the sediment samples of WBS and HR sediments. The end points of the test were biomass, lipid content and PCB concentrations in the tissue. Further, analyses and interpretations of the test results will be presented. This abstract does not necessarily reflect USEPA policy.

**TP072 Bioavailability of Heavy Metals in Two Earthworm Species and its Relationship to Extractable and Labile Metals in Soils** Y. Liu, Univ of California, Riverside, Environmental Sciences Dept, UC-Riverside, Environmental Sciences; D. Parker, Univ of California, Environmental Sciences Dept. The bioavailable pool of Cd and Zn to the earthworms *Eisenia fetida* (*E. fetida*) and *L. terrestris* (*L. terrestris*) in soils was investigated using stable isotopic dilution (SID) technique and chemical extraction methods. Ten soils used for earthworm culturing were from three sources with variety of soil properties. The soils were extracted by five chemical solutions and the amount of extractable Cd and Zn followed the order: EDTA>1M CaCl<sub>2</sub>>DTPA>0.05M Ca(NO<sub>3</sub>)<sub>2</sub>>0.01M Ca(NO<sub>3</sub>)<sub>2</sub>. In each extraction, the fraction of extractable-Cd was significantly higher than the extractable Zn. SID technique was used to get Cd, Zn labile pool (E-value) in soils. It was showed in average the fraction of labile Cd (44.7%) is significantly higher than that of Zn (25.4%), which indicated Cd is more mobile in soil compared to Zn. The labile pool of Cd and Zn was found to be independent with soil pH, total organic carbon and clay content. It was observed the accumulated Cd and Zn in two earthworms were highly correlated with heavy metals labile pool (p

**TP073 Impact of Solar Drying on PAHs and NPEs Removal in Sewage Sludge** N.A. Perendeci, Univ of Akdeniz, Dept Environmental Eng.; M. Barret, N. Delgenes, M. Pye, Laboratoire de Biotechnologie



de l'Environnement, INRA; V. Yilmaz, Univ of Akdeniz, Dept Environmental Eng.; H. Carrere, D. Patureau, Laboratoire de Biotechnologie de l'Environnement, INRA. Occurrence of priority and emerging organic contaminants in the aquatic environment is now undeniable. Sewage treatment plants (STPs) play a main role in their environmental dissemination, because it is a point of convergence of various type of water containing a variety of compounds present at low level. According to the extensive use of biological processes in urban WWTPs, many studies focused on the efficiency of biological treatments and the influence of the operating parameters associated to these systems to remove these contaminants. If it was demonstrated that their removal is related to their biodegradability, sorption is one of the main mechanisms that drive their fate in STP, particularly the hydrophobic ones. Indeed many studies focussed on the occurrence of contaminants in sludge. However, agricultural land application of sewage sludge from WWTP is one of the common ways for their disposal: the presence of such contaminants may thus limit their use on agricultural lands. Various sludge processes are used to stabilize the sludge before spreading. If anaerobic digestion and composting have received considerable attention for their impact on contaminants removal, other processes like solar drying have received little. In this study the effects of solar drying on nonylphenol polyethoxylates (NPEs), nonylphenol (NP) and 13 PAHs removal present in sewage sludge was evaluated. For this purpose, sewage sludge taken from two Turkish WWTPs (Hurma and Lara) was solar dried in controlled prototype greenhouse. NPEs, NP and PAH concentrations were monitored during the solar drying. Samples were extracted using accelerated solvent extractor at 120°C and 100 bar with hexane/acetone. NPEs, NP and PAHs analysis were carried out using reverse phase-HPLC coupled to fluorescence detector. NPEs, NP and PAHs were measured in the two sewage sludge samples at level commonly found in other European sludge. During the 120 hours solar drying of the two sludge samples, the NP concentration decreased sharply whereas only for Hurma, the NP2EO and NP1EO concentrations increased. The total PAH concentration in the sludge was 469.018 µg/kg<sub>TS</sub> and 303.527 µg/kg<sub>TS</sub> for respectively, Hurma and Lara. During the solar drying, all the PAHs concentrations fell down with 55 and 50 % removal for respectively, Hurma and Lara. As no biological activity was measured during the process, these contaminant removals are probably linked to volatilization, sequestration or photodegradation.

**TP074 Occurrence and Contribution to Global Contamination by Organohalogen and Trace Elements from India** M. Subramanian, G. Devanathan, N. Ha, Ehime Univ, Center for Marine Environmental Studies (CMES); A. Eguchi, Ehime Univ, Center for Marine Environmental Studies; K. Nomiyama, T. Itai, Ehime Univ, Center for Marine Environmental Studies (CMES); T. Isobe, Ehime Univ, Senior Research Fellow Center (SRFC); S. Takahashi, S. Tanabe, Ehime Univ, Center for Marine Environmental Studies (CMES). India is now confronting the huge problem of e-waste, both locally generated and internationally imported, posing a serious threat to human health and the environment. Our decades-long studies on samples of air, soil, fish, and also human hair, blood and milk collected from Mumbai, Bangalore, Chennai, Kolkata, Chidambaram, Parangipettai, Karaikudi and Sivakasi in India confirm the presence of polybrominated diphenyl ethers (PBDEs), hexachlorocyclododecanes (HBCDs), dioxins and related chemicals (DRCs), polychlorinated biphenyls (PCBs) and heavy metals in the Indian environment, at considerable levels. It has been noticed that the e-waste recycling activities in Bangalore and Chennai create elevated levels of PBDEs in the ambient air, which had also reflected in the soil from the same locations. Higher levels of HBCDs and brominated and mixed halogenated dioxins and furans were found in the soils from e-waste recycling areas than in non e-waste soils. Further, the scalp hair samples of the recycling workers contained higher levels of PBDEs and also PCBs and HBCDs indicating e-waste recycling activities in India as a source for human exposure to these contaminants. Fish collected from Mumbai, Chennai, Karaikudi and Sivakasi had considerable levels of PCBs, PBDEs and HBCDs, the levels of PCBs being higher than PBDEs and HBCDs. In addition, HBCDs were lower than PBDEs. A recent work from our center showed the presence of not only PBDEs and PCBs but also their metabolites, OH-PCBs, OH-PBDEs and MeO-PBDEs in serum of e-waste workers and general population in India indicating possible metabolism of these toxic chemicals in the animal bodies and/or the environment. Another interesting finding is the detection of increasing levels of PBDEs in Indian mothers' milk of the year 2009 than those gathered during 2006, revealing that e-waste related chemicals are increasing even in the general population

of India, while the chemicals like PCBs are decreasing. Apart from organic chemicals, some trace elements were higher in the air and consequently in the hair of the workers at the e-waste recycling sites than the control site. Higher Hazard Quotient values (HQ>1) observed for certain metals in the soil from the recycling areas, especially for children, who are highly susceptible, is of serious concern.

**TP075 Organohalogen Contaminants of Emerging Concern in Predatory Birds from Coastal South Carolina, USA** J.L. Reiner, National Institute of Standards and Technology, Environmental Health Sciences, NIST, Analytical Chemistry Division; S. Rossman, Michigan State Univ, Dept of Zoology; P. Ostrom, Michigan State Univ; S. Hughes, K. Bargnesi, J. Elliott, The Avian Conservation Center and Center for Birds of Prey; J. Yordy, National Institute of Standards and Technology, Hollings Marine Laboratory, Medical Univ of South Carolina, Marine Biomedicine and Environmental Sciences Program. As a result of being top predators, birds of prey are susceptible to bioaccumulating countless environmental contaminants. Since recent environmental surveys have shown the widespread occurrence of polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (PFCs) in the aquatic and terrestrial environment, the levels of these contaminants, in addition to legacy persistent organic pollutants (POPs), were measured in birds of prey livers from the coastal region of South Carolina (USA). The 26 birds included in the study comprised 10 species (red-tailed hawks, red-shouldered hawks, barred owls, great horned owls, eastern screech owls, Mississippi kites, turkey vultures, one black vulture, one osprey and one American kestrel), spanned 2 to 3 tropic levels (as determined by stable isotope analysis), exhibited a wide range of body conditions and utilized a diversity of habitats (rural or urban; terrestrial or marine), allowing for an assessment of the factors that influence PBDE and PFC exposure in predatory birds within this region. Despite their status of newly emerging contaminants, PFCs (95% CI, 75.9 ng/g wet mass to 168 ng/g wet mass) were within the same order of magnitude as legacy contaminant levels (PCBs 95% CI, 34.4 ng/g wet mass to 302 ng/g wet mass). In comparison, PBDEs (95%CI, 5.45 ng/g wet mass to 47.5 ng/g wet mass) were amongst the highest lipid-normalized levels reported for these compounds in wildlife to-date (maximum, 200 µg/g lipid). This presentation will discuss the implications of this exposure as well as relationships between exposure and feeding ecology, body condition, and proximity to human populations.

**TP076 Challenges in the Application of Mercury Environmental Quality Standards for Biota** D. Vignati, Irsa-Cnr, IRSA-CNR, UOS Brughiero; M.S. Bank, Harvard Univ, School of Public Health, Dept of Environmental Health, Harvard School of Public Health, Dept of Environmental Health. Environmental Quality Standards (EQS) for Hg in natural waters are among the lowest for all trace elements (in the range of ng L<sup>-1</sup>). Quantifying total Hg at such low levels is extremely difficult because of the contamination risks associated with sampling and sample handling prior to analytical measurements. Furthermore, the actual chemical species of concern is methylmercury (MeHg) whose routine quantification in monitoring programs is still far to come. EQS based total Hg concentration in biota (prey tissue) offer an attractive alternative to water-based standards for the protection of top predators against Hg secondary poisoning. Levels in biota are much higher (mg kg<sup>-1</sup>) than in water and measuring Hg in biota would automatically account for important confounding factors regulating Hg bioavailability. However, this second type of EQS generically refers to 'biota' without further specification for the trophic level or for any preferred taxonomic group. The current EU standard for biota (0.020 mg kg<sup>-1</sup>) would result in a quasi-total non-compliance if applied to fish; while application to filter-feeders (e.g., *Dreissena polymorpha*) or benthic organisms (e.g., chironomids and oligochaetes) would yield a much less worrying picture. This contribution will examine the current problems and possible solutions to facilitate switching the Hg regulation from the traditional water-based EQS to the more ecologically relevant biota-based ones.

**TP077 Mercury in Perch from the Scandinavian Region – Spatial and Temporal Trends** A. Miller, Naturhistoriska Riksmuseet; S. Danielsson, J. Hedman, A. Bignert, Naturhistoriska Riksmuseet, Dept of Contaminant Research; M. Verta; E. Fjeld; S. Rognerud; P. Porvari. To investigate trends in bioavailable mercury within the Scandinavian aquatic environment, mercury concentrations in European perch (*Perca fluviatilis*), a freshwater cyprinid, were collated from national monitoring programs within Norway,

Sweden and Finland to form a single database (n=6779). The perch came from reference lakes with no known local contaminant sources. Mercury concentrations were weight adjusted to 300, 200 and 100 g perch body weight. Geographical patterns and changes in mercury concentration in perch over time were examined. No statistically significant geographical trends were seen; however a comparison in mercury concentrations in perch pre- and post-1995 show a non-statistical geographical pattern, with increasing mercury concentrations seen in Norway and eastern Finland. The average mercury concentration in perch (adjusted to 300 g) was 500 ng/g ww, and did not differ significantly between countries. This implies that about half of the perch weighing 300 g caught in Scandinavia between 1968 – 2005 exceeded the current maximum allowable concentration of mercury in fish for human consumption in the EU of 500 ng/g ww. When mercury concentrations in perch taken from the most recent 10 years of data (1996 – 2005) were compared to the data set as a whole (1968 – 2005), it indicated that concentrations have been increasing in this latest period. Despite large reductions in mercury use and production in all three countries, mercury levels in perch continue to be higher in Scandinavia than other European areas. It has been suggested that background mercury concentrations in Scandinavia are generally higher than the rest of Europe, and this may need to be taken into account when considering mercury concentrations in fish for human consumption from this area.

**TP078 Quantitation of Organophosphate Insecticides in Drinking Water Using Automated Online Sample Preparation and a 3-D Ion Trap** J. Beck, M. Blackburn, J. Wang, M. PrietoConaway, Thermo Fisher Scientific. Organophosphates are irreversible acetylcholinesterase inhibitors and as such are highly toxic. Due to the toxicity of organophosphates and other effects such as delayed learning rates in children, an increased risk of Alzheimer's, and chronic fatigue symptoms it is essential to be able to detect even low levels of these compounds. This presentation will demonstrate the use of automated online sample preparation coupled with a 3-D iontrap mass spectrometer, performing full scan MS2, for the detection of organophosphates in drinking water. Calibration curves were generated from 1mL or 4mL injections of standards spike into HPLC grade water with no further preparation. Quantitation was based on the post acquisition extraction of multiple fragments, where possible, for each compound from the full scan MS2 data. Detection limits in the parts per trillion were achieved for most of the organophosphates analyzed. Several local water sources were also analyzed for the presence of organophosphate using the previously described method.

**TP079 Screening for Pharmaceuticals and Personal Care Products in Drinking Water Using a 3-D Iontrap and Automated Online Sample Preparation** J. Wang, M. Blackburn, J. Beck, M. PrietoConaway, Thermo Fisher Scientific. Pharmaceuticals and personal care products (PPCP) consist of human and veterinary drugs, consumer products such as sun-screens, nutritional products, and personal hygiene products. These compounds enter the environment through a variety of sources including wastewater effluent, landfill leachate, industrial effluent, and animal feed lots. Environmental impact of these compounds remains unclear. However, in 2007 the EPA released method 1694 addressing the analysis of these compounds. This method utilizes solid phase extraction (SPE) followed by LCMSMS monitoring a single transition. We will demonstrate an automated online sample preparation that eliminates the need for the laborious SPE method described in EPA method 1694. In addition, we will show automated quantitative and qualitative data processing using 3-D iontrap full scan data for quantitation and data dependent (DD) full scan MS2 spectra for confirmation of PPCPs. Standards were spiked into HPLC grade water and 1mL injections were analyzed with no additional preparation. The PPCPs were detected down to levels as low as 10ppt in spiked standards. Several sources of drinking water were analyzed and caffeine was quantitated in bottled beverages. Calibration, quantitation, and confirmation of PPCPs detected was simultaneously achieved applying an automated quan/qual software package designed for routine analysis in production laboratories.

**TP080 A Protocol for Measuring In Vivo Biotransformation Rates of Hydrophobic Substances in Fish** J. Lo, Simon Fraser Univ, Biological Sciences, Simon Fraser Univ, Dept of Biological Sciences, Simon Fraser Univ, M.E.T. (Master of Environmental Toxicology) student; C.J. Kennedy, Simon Fraser Univ, Dept of Biological Sciences; S.V. Otton, Simon Fraser Univ; M.M. Moore, Simon Fraser Univ, Biological Sciences Dept; F.A. Gobas,

Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment), Simon Fraser Univ. It is well recognized that biotransformation is an important process controlling the degree to which chemicals can bioaccumulate in biota. However, there are no standardized methods to measure biotransformation rates and methods for the assessment of biotransformation rates are at an early stage of development, largely because of the lack of good quality data on biotransformation rates that could be used to develop or test biotransformation rate assessment methods. In this paper, we propose a methodology for the empirical determination of biotransformation rates and test this methodology in bioaccumulation studies in rainbow trout (*Oncorhynchus mykiss*) involving fifteen chemicals, including metabolizable chemicals of very high K<sub>ow</sub>. We report biotransformation rates in the liver, intestinal tract, carcass and the whole organism basis to test the ability of in vitro biotransformation assays to assess in vivo biotransformation rates. Results show that non-metabolizable PCB and chlorobenzene congeners have lower elimination rate constants compared with test compounds with comparable hydrophobic properties. We conclude that this study presents a promising method for determining empirical in-vivo metabolic biotransformation rates in fish. Results from this study should also help in the extrapolation of in vitro to in vivo biotransformation rate constants.

**TP081 A Sensitivity Analysis Approach to Bioaccumulation and Biomagnification in Aquatic Food Webs** D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101); F.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment), Simon Fraser Univ; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute; R.M. Seston, K.B. Woodburn, Dow Corning Corporation, Health & Environmental Sciences. The potential of a chemical substance to accumulate in aquatic organisms and to increase in concentration with increasing trophic level is a criterion used to classify substances as being bioaccumulative in the environment. For aquatic organisms, bioaccumulation factors (BAFs) describe the accumulation of contaminants by living organisms relative to the amount stored in the water compartment. Similarly, biota-sediment accumulation factors (BSAFs) describe the accumulation of contaminants by living organisms relative to the amount stored in the sediment compartment. Biomagnification factors (BMFs) describe the increase in concentration of a substance in living organisms that are separated by a single trophic level step on a food chain. Because of biomagnification processes, individual BAF or BSAF values are dependent upon trophic level position of the organism under consideration. Consequently, trophic magnification factors (TMFs), which describe the increase in concentration of a substance in living organisms that occupy successively higher trophic levels within a food web, are used to assess bioaccumulation and biomagnification of chemicals in the environment. This presentation will show how these field metrics for assessment of bioaccumulation and biomagnification (i.e., BAF, BSAF, BMF, and TMF) are interrelated. We will also use actual field data and a model food web to evaluate various approaches that may be used to calculate bioaccumulation and biomagnification metrics from measured concentrations, normalized concentrations (for example, to lipid content), concentration ratios, and reference materials that are known to bioaccumulate and biomagnify (for example, polychlorinated biphenyls). Several factors may directly affect the relationship between trophic position and the bioaccumulation and biomagnification of contaminants in aquatic ecosystems. Therefore, the evaluation will include an assessment of the sensitivity of the different approaches to parameters such as trophic level, nitrogen-15 enrichment factors (used to estimate relative trophic level position), food-chain outliers, unbalanced designs, omnivorous feeding, and lipid content.

**TP082 Application of Fish In Vitro Metabolism Assay for Assessing Bioaccumulation Potential** A. Adekola, Maxxam Analytics, Graduate student in Masters in Environmental Toxicology; C.V. Eickhoff, Maxxam Analytics, Ecotoxicology. Currently, bioaccumulation potential is assessed in silico using models such as BCFBAF or in vivo using the OECD 305 test. Inherent metabolic potential is one of the most important factors affecting the bioaccumulation of chemicals in aquatic organisms. The ILSI HESI Bioaccumulation Group has developed a combined approach for estimating the biotransformation rates of potentially bioaccumulative chemicals using an

vitro fish S9 metabolism assay and a predictive model (Cowan-Ellsberry et al., 2008). We evaluated the in vitro method by incubating environmentally relevant chemicals such as pyrene, 4-nonylphenol, fluoxypyr methyl heptyl ester, methoxychlor and a synthetic pyrethroid with the S9 fraction isolated from rainbow trout strains. When statistically significant, clearance rates obtained from the in vitro assay were extrapolated to whole fish biotransformation rates ( $k_{met}$ ) and used to refine BCF computer model predictions based on log  $K_{ow}$ , molecular weight and structure alone. The BCF values estimated from this combined approach are similar to the in vivo BCF values found in literature for pyrene (Carlson et al. 1979, Ogata et al. 1984, Jonsson et al. 2004) and 4-nonylphenol (Ekelund et al. 1990, Ahel et al. 1993, Snyder et al. 2001). This study shows that the combined in vitro and in silico approach can be effective for predicting BCF values. However, the metabolic potential of chemicals represented by the  $k_{met}$  value can also be used to assess bioaccumulation potential in relative terms. Funding was provided by the European Commission JRC/IHCP/In Vitro Toxicology Unit – ECVAM, Contract #CCR.IHCP.C434207.X0 and by the European Chemical Industry Council Long-range Research Initiative (CEFIC-LRI), Contract #LRI-ECO6.2-ILSIHESI-0804.

**TP083 Empirical Evidence and Ecological Support for a Strong Water Column Tie in Hudson River Benthic Fish** E. Lamoureux, J. Connolly, D. Chiavelli, Anchor QEA, LLC. A significant source of uncertainty in bioaccumulation modeling is the relative proportion of contaminants derived from the water column and the "bioavailable" sediment. Bioaccumulation models typically deal with this issue by incorporating the results of invertebrate surveys and fish gut content analyses to characterize the proportion of invertebrate water column feeders and benthic detritivores that forage fish consume. Polychlorinated biphenyl (PCB) body burdens in water column and benthic invertebrates are then respectively modeled as resulting from exposure to contaminants associated with water column particulates, or with bulk sediments averaged over a depth typically in the range of 2 to 15 cm. However, this approach is at odds with research demonstrating that benthic invertebrate growth and habitat selection is strongly correlated with the availability of fresh organic material. Because the bioavailable sediments typically have an average age of multiple years, the organic matter is mostly refractory in nature. The contaminants associated with this material generally have relatively low bioavailability because a major fraction is resistantly sorbed; a significant body of literature supports the notion that resistant sorption of contaminants limits their bioavailability and that the level of resistance increases with the length of time the contaminant has been associated with the sediments. Based on this research, contaminant body burdens in benthic invertebrates should be more strongly tied to fresh organic matter recently derived from the water column than to bulk sediment organic matter. This idea is supported by the spatial patterns of PCB concentrations in Hudson River invertebrates and fish: benthic fish tissue and benthic invertebrate PCB patterns track water column PCB patterns, decreasing by a factor of less than 2 moving from upstream to downstream, whereas surface sediment PCBs decrease by almost a factor of 10. Thus, the empirical evidence is that Hudson River benthic fish PCB exposure is more strongly linked to water column particulate PCB concentrations than to the bulk sediment PCB concentrations, and that relying solely on a bulk sediment PCB exposure to model PCB concentrations in benthic invertebrates may be inaccurate. Efforts to model bioaccumulation in the Hudson indicate that sediment sources to benthic invertebrates cannot be ignored, but that water column sources must be accounted for as well.

**TP084 Modeling Bioconcentration Factor (BCF) of Many Organic Compounds in Fishes** D.T. Kuo, Univ of Delaware, Dept of Civil and Environmental Engineering; D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering. Fishes occupy an important ecological role in aquatic environments. They are also a significant source of our dietary intake. As a result, the prediction of bioconcentration factor (BCF) of current or new organic compounds in fish has been of great interest to the public, regulatory agents, and scientists. BCF is measured by chronically exposing individual fish to test chemical under well-controlled laboratory conditions. It can be an important component of models that assess the likelihood of a chemical to bioaccumulate in fish in their natural habitat. Despite the improvement and standardization of the BCF methodology and the accumulation of BCF measurements for many chemicals and fish species over the years, researchers have constructed prediction models based on very different conceptualization of the bioaccumulation processes and mechanism. Furthermore, most

current models contain model coefficients that are determined 'after-the-fact' via regression and/or correction schemes. We have re-analyzed bioconcentration data by considering only chemical partitioning with or without biodegradation/biotransformation kinetics. The predictive performance of our BCF model was comparable to those of the others. Our analysis and model results suggest that: (1) fish BCFs data can be predicted without explicit after-the-fact fitting of the model parameters, and (2) future model improvement should be approached by improving our predictive capabilities in chemical partitioning and biodegradation kinetics, and (3) elaborate parameter estimation schemes may be unnecessary.

#### **TP085 Moving Bioaccumulation Assessments to the Next Level:**

**Progress Made and Challenges Ahead** C.E. Cowan-Ellsberry, CE2 Consulting, LLC, Central Product Safety, CE2 Consulting; M. Bernhard, Procter & Gamble; M. Bonnell, Environment Canada, New Substances Division, Environment Canada, Science and Risk Assessment Directorate; J. Domoradzki, Dow Corning Corporation, Senior Toxicology Specialist; M.R. Embry, ILSI Health and Environmental Sciences Institute, ILSI Health & Environmental Sciences Institute (HESI), Senior Scientific Program Manager; S. Erhardt, Michigan State Univ, TERC; M. Halder, European Commission, Joint Research Centre, ECVAM; J.W. Nichols, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; H. Segner, Univ of Bern, Centre for Fish + Wildlife Health. Since 2005, the HESI Bioaccumulation Project Committee has helped to advance the science of bioaccumulation ('B') in several areas: developing in vitro methods as part of weight of evidence approaches to 'B' assessment; publishing peer reviewed papers on the state of 'B' science; advancing models to better screen organic chemicals for 'B'; and funding research in basic fish physiology to better understand ADME processes. In addition, the HESI committee has collaborated with SETAC and others to sponsor workshops bringing together academia, industry and regulatory authorities to discuss and advance 'B' science. In February 2011, a workshop was held to highlight recent and ongoing research efforts funded by HESI and others, to demonstrate progress that has been made in the bioaccumulation field, to identify key gaps in current 'B' knowledge, and to discuss obstacles that hinder implementation of this recent progress into regulatory assessments. The ultimate goal of this workshop was to provide guidance to the HESI 'B' Project Committee to further develop the research program and move the science of bioaccumulation forward. Participants included 65 academic, industry and regulatory scientists from 14 countries on 4 continents. The workshop included introductory presentations of the current research, breakout sessions to discuss the research and identify needed work, and finally an assimilation session where the key leanings and next steps were captured. For the discussions, the participants focused on four areas of bioaccumulation research: in silico, in vitro, in vivo and field. In addition to the focus on the research and science, one session discussed the forming weight of evidence for bioaccumulation and obstacles for regulatory acceptance of new methods. The participants emphasized the importance of the recent and on-going research but recommended that the committee seek to move from a linear tiered approach to a network linked approach with cross-cutting focus areas. These cross-cutting focus areas included: 1) Improve overall data access; 2) Bridge in silico – in vitro – in vivo – field information; 3) Develop benchmarks for the available bioaccumulation methodologies; 4) Address issues related to uncertainty; and 5) Engage in a continued regulatory dialog. This presentation will focus on describing the outcomes of the workshop and the follow-on activities.

**TP086 Bioamplification as a Non-steady State Bioaccumulation Mechanism for Persistent Organic Pollutants (POPs) in Fish and Wildlife** J.M. Daley, Univ of Windsor, Great Lakes Institute of Environmental Research; K.G. Drouillard, Univ of Windsor, Great Lakes Institute for Environmental Research. Persistent organic pollutant (POP) bioaccumulation models have been generally formulated to predict two main processes, bioconcentration and biomagnification under a steady-state framework. A third bioaccumulation process that can mediate chemical potential in an organism is bioamplification. Bioamplification occurs when an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate contaminants. It is explicitly a non-steady state process that arises from sudden changes in steady state to non-steady state conditions or from perturbations of the non-steady state trajectory. Although bioamplification is not generally recognized as a bioaccumulation process independent of biomagnification, the consequences of bioamplification can be important.



Bioamplification generally occurs under specific life history stages when an organism experiences major bioenergetic bottlenecks. Examples of bioamplification in a wide variety of species including bird and fish embryos during egg development, overwintering yellow perch, larval Chinook salmon, amphibians during tadpole-frog metamorphosis and emergent aquatic insects will be presented. These examples will demonstrate that bioamplification is a general bioaccumulation process that contributes to enhanced chemical fugacities of POPs in a wide variety of animal species.

**TP087 Challenges in Evaluating Biomagnification in Terrestrial Food Webs: A Study with Perfluorinated Compounds** C. Mueller, ETH

Zurich, Institute for Chemical- and Bioengineering, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Analytical Chemistry; A.O. De Silva, Environment Canada, Water Science & Technology Directorate, Environment Canada, Water Science and Technology; X. Wang, Environment Canada, Aquatic Ecosystem Protection Research Division; A.D. Morris, Univ of Guelph, Environmental Biology, Environment Canada, Environment Canada, graduate student; M. Gamberg, Gamberg Consulting; D. Muir, Environment Canada, Aquatic Ecosystem Protection Research Division, Environment Canada, National Water Research Institute. Assessing biomagnification of organic contaminants is a difficult task due to the need to understand properties of the chemicals and characteristics of the food web. It is even more complex for perfluorinated compounds (PFCs), e.g., perfluorinated carboxylates and sulfonates because their mechanism of accumulation seems to differ from classic lipophilic chemicals. PFCs are considered proteinophilic and accumulate mainly in protein rich compartments such as liver and blood. Little is known about PFC bioaccumulation in terrestrial organisms. Therefore, we investigated biomagnification of PFCs in a terrestrial food web in two areas in the Canadian Arctic. This food web included vegetation (plants and lichens), barren ground caribou and wolves. There are certain advantages in studying such remote food webs: The lichen-caribou-wolf food web has been well studied and it is relatively simple as caribou feed mostly on lichen and wolves living near caribou herds almost exclusively feed on them. Therefore, it is potentially easier to assess diet-consumer relationships than for complex aquatic food webs. PFCs were measured for all species (with muscle, kidney and liver for caribou and wolves) along with stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in muscle) to assess biomagnification (BMF) and trophic magnification factors (TMF). To determine BMFs and TMFs for PFCs two alternate calculation methods were tested: (i) calculation on the basis of the liver concentration or (ii) calculation on the basis of a theoretical whole body concentration. Both approaches have advantages but also carry potential errors: (i) is simple to calculate but may overestimate factors due to higher concentrations in caribou and wolf liver and ignores that different tissues may have different accumulation behavior. (ii) may represent more realistic factors but also carry higher uncertainty due to necessary estimations of unknown body fractions. Utilizing the TMF approach to terrestrial food webs has additional challenges which apply not only to PFCs but also other persistent, bioaccumulative organics. Especially seasonal variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in vegetation in temperate and arctic climates as well dietary variation in herbivores may alter TMFs. Selection of the species used to calculate trophic levels and of the appropriate trophic enrichment factor are therefore important factors which need further study in terrestrial food webs.

**TP088 Effect of Different Extraction Procedures for the Determination of Aqueous Analyte Concentrations on the Result of Fish Bioconcentration Studies** C. Schlechtriem, Fraunhofer IME, Oekotoxikologie;

L. Boehm, R. Duering, Institute of Soil Science and Soil Conservation, Justus Liebig Univ Giessen. Measured bioconcentration factors (BCF) and n-octanol-water partition coefficients (Kow) of hydrophobic organic chemicals (HOC) show a curvilinear relationship up to  $\log \text{KOW } 5-6$ . BCF values of more lipophilic substances tend to level off or decline (hydrophobicity cutoff). Debated reasons are steric effects, but also measurement artefacts. Jonker and van der Heijden (2007; Environ Sci Technol 41:7363) suggested that the overestimation of bioavailable aqueous HOC by the presence of non-bioavailable HOC bound to dissolved organic matter, might lead to an underestimation of the true uptake. This was supported by the determination of freely dissolved aqueous concentrations by solid-phase microextraction (SPME). The suitability of SPME to measure highly hydrophobic substances is described in the literature. However, the standard method for sample preparation in BCF-tests according to OECD TG 305 is liquid-liquid-extraction (LLE). The aim of this study was to investigate the

potential of SPME with regard to the determination of aqueous analyte concentrations in fish BCF studies with special reference to different contents of organic matter (OM). Water with different concentrations of organic matter was spiked with four test substances ( $\log \text{KOW} > 5$ ) and analyzed at equilibrium conditions. Analysis of samples with SPME was compared to conventional sample preparation with LLE. With SPME, the detection of freely dissolved analyte concentrations is possible, as is the determination of sorption. Extraction of samples with LLE yields total analyte concentrations. Results show, that the freely dissolved concentration of highly hydrophobic analytes (which corresponds to the bioavailable fraction) is significantly reduced due to sorption to OM – already beneath the permitted concentration of 2 mg L<sup>-1</sup> TOC in the dilution water according to OECD TG 305. The results are compared with the outcome of a flow-through fish test on rainbow trout which was carried out to investigate the effect of different extraction procedures on the result of BCF studies carried out under realistic experimental conditions. The results show that SPME can give important information on the ratio between bound and freely dissolved compounds and help to estimate suitable TOC for highly lipophilic substances prior to a BCF study. However, SPME measurements should not replace LLE procedures to keep the results of BCF studies comparable.

**TP089 Laboratory Biotransformation – A Link in Understanding Field Bioaccumulation?** P. Leonards, Institute for Environmental Studies, VU

Univ, Chemistry & Biology, VU Univ, Institute for Environmental Studies; S.v. Leeuwen, B.v. Hattum, VU Univ, Institute for Environmental Studies; F. Gobas, Simon Fraser Univ; H. Leslie, VU Univ, Institute for Environmental Studies. The current state of bioaccumulation science relies heavily on fish bioconcentration (BCF) testing according to international protocols in order to predict the level of bioaccumulation of chemicals in real organisms in food webs in the field. Biotransformation is a key metric in bioaccumulation. The presence of metabolic enzyme systems (CYP450 types), induction of enzyme systems (concentration-dependency), and metabolisation rates are important aspects that determine the overall biotransformation rate – and bioaccumulation – of a chemical. How similar is biotransformation in the laboratory and in the field? The test species in BCF studies often are kept at high temperature (about 25°C), while in the field situation temperatures can be much lower, resulting in lower metabolic rates and higher bioaccumulation. How easily can we use laboratory biotransformation rates to predict bioaccumulation in the field? We present here examples of how laboratory biotransformation of chemicals, with a wide range of  $K_{\text{oa}}$  and  $K_{\text{ow}}$ , are related to bioaccumulation and biomagnification in food chains, and to model biotransformation predictions. Laboratory biotransformation rates of the chemicals were studied using S9 rat liver incubation studies. From a pelagic and benthic food chain in the Western Scheldt estuary (The Netherlands) field derived biotransformation rates of the chemicals were estimated using relative concentrations compared to a persistent chemical (PCB153) in predator/prey interactions, including higher trophic organisms (piscivorous bird). The laboratory biotransformation rates were related to the estimated relative field rates and model predictions of biotransformation rates in fish. These biotransformation data were then examined in light of the trophic magnification factors (TMF) measured in this ecosystem. We concluded that the chemicals can be grouped in a persistent and a non-persistent group based on laboratory and field data. These data will be used to develop and test models that describe the relationship between in vitro biotransformation rate constants, in-vivo bioconcentration factors and in situ bioaccumulation in food-webs.

**TP090 Patterns of Bioaccumulation of Lake Huron Lake Trout**

(*Salvelinus namaycush*) M. Ryder, D. Haffner, Univ of Windsor, GLIER. Steady state kinetics has been the primary assumption when describing the accumulation of organic contaminants, including super hydrophobic compounds ( $\log \text{Kow} > 6$ ). Recent studies have shown that not all chemicals achieve a steady state within an organism's lifetime. This study investigates the accumulation of polychlorinated biphenyls (PCB's) in lake trout (*Salvelinus namaycush*) from Lake Huron and tests the assumption these organisms achieve a steady state in their lifetime. Micro-extraction methods were used to extract both lipids and 24 individual PCB congeners from 36 lake trout. Whole body homogenates were used to provide a better representation of total body burdens. Fish ranged in ages from 3 to 11 years. Sums of the lipid normalized concentrations of the 24 congeners in the fish ranged from 610.7 to 2692.9 ng/g. Preliminary results indicate that compounds of high hydrophobicity ( $\log \text{Kow} > 6.5$ ) were less likely to achieve steady state than

low Kow compounds, and the super-hydrophobic chemicals continued to accumulate throughout the life time of these fish. These data suggests using a steady model can underestimate the level of contaminants present in the fish and therefore under predict the potential hazard associated with fish consumption guidelines.

**TP091 The Suitability of Freshwater Amphipods as Test Organisms for Bioaccumulation Studies** C. Schlechtriem, Fraunhofer IME, Oekotoxikologie; I. Goeritz, Fraunhofer IME; C. Schaefer, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Dept of Ecotoxicology. The bioaccumulation of sediment-associated contaminants by benthic invertebrates can be assessed by using the bioaccumulation test TGD OECD 315. Endobenthic aquatic oligochaetes burrow in the sediment and are therefore exposed to contaminants via multiple uptake routes including direct dermal contact, ingestion of contaminated sediment particles, porewater, and overlying water. Therefore, the test endpoint is defined as a bioaccumulation factor (BAF). However, the ultimate decisive bioaccumulation-criterion as part of the REACH regulation (Annex XIII) is the bioconcentration factor (BCF) reflecting the uptake of a test substance from the contaminated surrounding medium. A suitable method to deduce BCFs from BAFs derived from OECD 315 studies is still missing leading to a limited value of this test from the regulatory point of view. The aim of this study was to investigate whether benthic amphipods can be used as alternative test organisms for bioaccumulation studies, providing the opportunity to explain bioaccumulation from water (bioconcentration) and food (biomagnification) separately. In a first approach, an algal based diet suitable to grow the freshwater amphipods *Hyalella azteca* and *Gammarus pulex* was identified using a simple filter feeding system. The selected dietary component was then enriched with a highly lipophilic test item ( $\log P = 5$ ) and applied in a feeding study on both species. In a further approach animals were exposed to a test item of moderate lipophilicity ( $\log P = 3$ ) via the water. Animals collected during the bioaccumulation studies were analysed for their tissue concentrations. Based on the kinetic study design the depuration and uptake rates for both test items were determined which were further used to calculate species specific BCFs or biomagnification factors (BMF). The results of all studies are presented and compared with BCF and BMF values obtained from fish bioaccumulation studies carried out according to the revised TGD OECD 305. The potential of freshwater amphipods as test organisms for bioaccumulation studies is discussed.

**TP093 How Sources of Organic Matter Affect Mercury Concentrations in Stream Biota** T. Jardine, Griffith Univ, Australian Rivers Institute; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; J. Rasmussen, Univ of Lethbridge. Our understanding of the trophic transfer of mercury (Hg) through aquatic food webs has been advanced by including measures of stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) to assess trophic position and carbon flow, respectively. Numerous studies of lakes have shown that Hg concentrations in food web organisms are positively related to their relative trophic position and that this Hg vs.  $\delta^{15}\text{N}$  relationship can be used to contrast systems that have different biological, physical and chemical characteristics. In contrast to lakes, mercury biomagnification through riverine food webs is not well understood and is confounded by the dynamic nature of these systems and the complex reliance of these biota on in-stream or terrestrial carbon sources. Here we sampled two species of fish (blacknose dace, *Rhinichthys atratulus*; brook trout, *Salvelinus fontinalis*), several invertebrate taxa, periphyton and terrestrial producers of 60 temperate streams in New Brunswick, Canada. We used a gradient approach – a comparison of the strength of association between consumer isotope ratios and periphyton isotope ratios – across temperate streams that range in their pH to assess consumer reliance on terrestrial versus aquatic organic matter, and whether Hg concentrations in fish and their prey were related to these energy sources. Taxa varied in their use of terrestrial material, with grazing mayflies (Heptageniidae), predatory stoneflies (Perlidae), one species of water strider (*Metabates hesperius*) and the fish blacknose dace (*Rhinichthys atratulus*) having strong connections to aquatic sources, while water striders (*Aquarius remigis*) and brook trout (*Salvelinus fontinalis*) showed a weak link to aquatic food sources. In addition, stream pH – known to affect Hg concentrations in lake biota – was a much better predictor of Hg in predatory invertebrates that relied mainly on aquatic carbon sources when compared to those that used terrestrial carbon. These results suggest that it is important to understand energy sources and flows in these food webs before examining

the importance of water chemistry or other variables on Hg concentrations and fate in riverine food webs.

**TP094 Biogeochemical Cycle of Mercury in Urban Streams** A. Aragon-Jose, J. Bushey, Univ of Connecticut, Dept of Civil and Environmental Engineering; C. Perkins, Univ of Connecticut, Center for Environmental Sciences and Engineering; M. Mendes, Univ of Connecticut, Dept of Civil and Environmental Engineering; G. Ulatowsky, Univ of Connecticut, Center for Environmental Sciences and Engineering. Mercury (Hg) can easily bioaccumulate in the food chain representing a risk even at low levels due to its high toxicity. The presence of urban activities can substantially alter different mechanisms and processes of the Hg biogeochemical cycle. Urban watersheds are characterized by high imperviousness and some may even be impacted by combined sewer overflows, both being fundamental factors contributing to Hg loading, mobilization, and shifts in bioavailability in urban watersheds. Research is still needed to understand the fate and dynamics of Hg in urban streams. We are collecting stream water and suspended sediment samples in the North Park River watershed in Hartford, CT (USA) during baseflow and precipitation events to assess potential for loading, mobilization, and bioavailability of Hg. We also collect water samples at the Hartford's waste water treatment plant (WWTP) from the influent, effluent, and the wet-weather lagoon. Water samples are analyzed for total, dissolved, and particulate Hg and methyl Hg (MeHg); major ions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{PO}_4^{3-}$ ); total suspended solids (TSS); and dissolved organic carbon (DOC). Our results show that both total and dissolved Hg concentrations increase in the streams during precipitation events, however the greatest portion of Hg is associated, and consequently transported, with suspended sediments, as evidenced by the high correlation coefficient ( $R^2 \sim 0.80$ ) between TSS and total Hg. No significant correlation was observed between dissolved or total Hg and DOC for the stream samples. In the WWTP, concentrations of both Hg and DOC are higher than those from the streams, but no significant correlation between TSS and Hg was observed, indicating differences in the transport mechanisms, and possibly on Hg sources between the streams and the WWTP. Overall, effluent samples exhibit lower Hg concentrations than their corresponding influent samples showing that Hg is somehow removed during the water treatment process. Unfiltered MeHg stream samples exhibited a similar pattern than that of total Hg, that is, an initial increase in concentration up to a maximum followed by a decrease close to the starting point. Dissolved MeHg is mostly below our detection limit for the stream sites. MeHg concentrations are generally higher at the WWTP compared to the stream. Both the influent and the lagoon sites show a decrease in MeHg concentration, whereas the effluent samples increase over time.

**TP095 Sulfur and Carbon Controls on Methylmercury in St. Louis River Estuary Sediment** N. Johnson, B. Beck, Univ of Minnesota Duluth, Civil Engineering. Transformation of inorganic mercury to methylmercury represents a public health concern due to its bioaccumulative properties in fish and neurotoxicity in humans. Biological mercury methylation primarily occurs in anoxic environments and has been shown to be limited by organic carbon and sulfate in wetland and saltwater estuary sediments. The work presented here investigates the controls on methylation in the St. Louis River system, a freshwater estuary potentially impacted by upstream mining. Surface water sulfate concentrations in the St. Louis River Estuary are impacted by both upstream mining and local urban sources and occur in a range that has been considered ideal for methyl mercury production (10-50 mg/L). Both in situ measurements and microcosm studies with sulfate amendments are being used to characterize biogeochemistry related to mercury methylation in different habitat zones of the St. Louis Estuary. Sediment phase methyl mercury was measured in the context of various biogeochemical parameters including byproducts of microbial metabolism ( $\text{H}_2\text{S}$ ,  $\text{Mn(II)}$  and  $\text{Fe(II)}$ ), total organic carbon, sulfate, and sulfate reducing bacterial abundance (qPCR). Results from intact sediment cores show that surficial sediment from each of four habitat zones has the capacity to methylate mercury (47-157 pg/g) and observed differences are interpreted in light of simultaneous geochemical measurements. Higher methylmercury concentrations in relatively low-organic habitat near the estuary mouth suggest that rates of biological sulfate reduction alone may not be the limiting factor for methylation. Microcosms with controlled sulfate concentrations in the overlying water are presently being used to examine the extent to which changes in sulfate loads will affect sediment biogeochemistry and associated mercury methylation. Preliminary results from microcosms imply that both

sulfate and organic carbon play a role in limiting methylation in different habitats within the estuary. A simple model for sediment methylmercury is used to explain observations and tie results to upstream sulfur management objectives.

**TP096 Methyl Mercury in Fish Tissue and the Water Column of the Upper Ohio River** D. Langseth, C. Tuit, Gradient. In 2001, the US Environmental Protection Agency (USEPA) changed the mercury ambient water quality criteria related to human health protection to be based on fish tissue concentrations, rather than water column concentrations. In 2009, USEPA issued guidance on how to incorporate the fish tissue criteria into discharge permitting under the Clean Water Act. The 2009 USEPA guidance describes two fundamental approaches, one based on only fish tissue, without translation to water column or discharge criteria values, and a second that includes translation to water column and/or effluent concentration values. Under the second option, the 2009 USEPA guidance recommends translation between water column and fish tissue concentrations based on measured values of methylmercury in fish tissue and the water column, preferably filtered water sample concentrations, for the location of interest. The Ohio River Valley Sanitation Commission (ORSANCO) has been collecting water column and fish tissue data from the Ohio River for several years, but until 2010 these samples were analyzed only for total mercury, and in water samples only for whole water samples. During 2010 and 2011, Gradient collected fish tissue and water column samples, both filtered and unfiltered, from the upper Ohio River. These samples were analysed for total and methyl mercury and provide some insight into mercury bioaccumulation in the Upper Ohio River.

**TP097 Hormetic Responses in *Daphnia magna* Growth and Reproduction Upon Exposure to the Energetic Compounds TNT, RDX, and the New Tetrazole Energetic TAGMNT** J.K. Stanley, US Army Engineer Research and Development Center, Environmental Laboratory; J.G. Sims, US Army Engineer Research and Development Center, Environmental Lab (EP-R); P. Chappell, Badger Technical Services; L.L. Escalon, US Army Engineer Research and Development Center, Environmental Laboratory; T. Habib, Badger Technical Services; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division; A.L. Russell, Badger Technical Services; A.J. Bednar, US Army Engineer Research and Development Center; M.A. Nascarella, Gradient. Currently, there is little information as to how munitions might cause effects when at low concentrations. A number of chemicals have been observed to cause a hormetic, or stimulatory, effect on animals exposed at low concentrations. The purpose of the present study was to identify the existence and the concentration range of hormetic responses observed for representatives of three different classes of energetic compounds. We used the model aquatic invertebrate *Daphnia magna* and assessed effects in 21-day growth and reproductive bioassays. The energetics assessed included hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), trinitrotoluene (TNT), and a newly developed nitrogen-rich tetrazole energetic triaminoguanidinium 1-methyl-5-nitriminotetrazolate (TAGMNT). Observed hormetic responses included increases in *D. magna* reproduction at TNT concentrations ranging from 0.06 to 0.22 mg/L and increases in *D. magna* growth at TNT concentrations ranging from 0.004 to 0.97 mg/L. Apparent, yet non-significant, increases in *D. magna* reproduction and growth were observed at RDX concentrations ranging from 0.02 mg/L to 14 mg/L and in growth of *D. magna* exposed to TAGMNT at concentrations ranging from 0.0002 to 0.01 mg/L. Ongoing efforts to be presented include an assessment of the magnitude and width of stimulation and work to elucidate the mechanism of the observed hormetic responses in *D. magna* using gene microarrays.

**TP098 Do Nanomaterials Used in Munition Formulations Alter the Fate of Energetic and Metal Munitions Constituents?** J.K. Stanley, US Army Engineer Research and Development Center, Environmental Laboratory; A.J. Kennedy, US Army Engineer Research and Development Center; A.J. Bednar, US Army Engineer Research and Development Center; A. Poda, US Army Engineer Research and Development Center; J. Brame, Rice Univ. While the environmental implications of energetic compounds and metals of military concern have received study, little is known about how the bioavailability of these military relevant compounds will be affected by their interaction with Army performance-enhancing nanomaterials. The likelihood of such interactions in the environment is high as nanomaterials

such as nano aluminum and carbon nanotubes (CNTs) are already used in energetic formulations as oxidizers and stabilizers, respectfully, and CNTs are also being investigated as components of battlefield obscurants. Such applications of nanomaterials are predicted to increase in the future. Additionally, unintended interactions between nanomaterial waste streams and contaminants of military concern (e.g., runoff from training ranges) are likely. The purpose of the present research was to determine the ability of nano aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) and CNTs to bind to and remove the munition constituents RDX (cyclotrimethylenetrinitramine) and tungsten (W) from solution as a first step in determining the bioavailability implications of such interactions. Al<sub>2</sub>O<sub>3</sub> size (11 – 1,500 nm) and CNT functionalization (hydroxylated) were varied to assess the effects of surface area and charge on this binding. Preliminary results from this ongoing work indicate significant reductions in aqueous RDX concentration in the presence of 100 mg/L nano Al<sub>2</sub>O<sub>3</sub> and apparent, albeit nonsignificant, reductions in RDX concentration in the presence of 100 mg/L multi-walled CNTs. Additional research to be presented includes assessments of binding strength between munition constituents and nanomaterials via thermally programmed desorption and the ability of nano Al<sub>2</sub>O<sub>3</sub> and carbon nanotubes to bind tungsten in solution. Bioavailability implications of such interactions will be directly measured using bioassays in upcoming years of this project. Consideration will be given to both bioavailability of the munition remaining in the water column and whether munition initially bound to nanomaterial is bioavailable following bio-uptake.

**TP099 Determining Diffusion Kinetics of Lithium Bromide Tracer into Nitrocellulose as a Distinguishing-Model for Nitroglycerin and 2,4-Dinitrotoluene** M. Simini, US Army Edgewood Chemical Biological Center, Environmental Toxicology, RDCB-DRT-E, E5641; R. Checkai, US Army Edgewood Chemical Biological Center; M. Minyard, US Army Defense Threat Reduction Agency; R.G. Kuperman, US Army Edgewood Chemical Biological Center, Environmental Toxicology; H.E. Allen, Univ of Delaware, Civil & Environmental Engineering Dept; D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering. Development of effective technologies for predicting the fate and transport of munitions constituents (MC) at explosives-contaminated sites is needed to sustain operational training and testing ranges. Dissolution of MC from propellant particles is a major concern at these sites. The presence of the nitrocellulose (NC) matrix complicates modeling of MC release from propellant and into soil solution. In addition to the dissolution of the soluble components, the role of adsorption to NC, and desorption from NC, matrix swelling in the presence of water, and diffusion within the matrix, all need to be taken into account. No adsorption/desorption kinetic model of MC into the NC matrix currently exists. The present study investigated the diffusion kinetics of lithium bromide (LiBr) into the NC matrix, using LiBr as an inert tracer in order to distinguish diffusion from adsorption and desorption of the MCs nitroglycerin (NG) and 2,4-dinitrotoluene (2,4-DNT) on NC. Experiments were conducted in triplicate using NC (12.3% N) pre-moistened for 5 days to a previously determined saturation point. A LiBr solution was added to determine rates of Li and Br diffusion into the NC matrix. Replicated samples were extracted from the solution at timed intervals from 0 to 48h, and analyzed for Li and Br simultaneously by ion chromatography. Diffusion of LiBr from the NC matrix into aqueous solution was also investigated; for this, NC was pre-saturated with LiBr and its diffusion into clean ASTM Type I water was determined as a function of time. Most diffusion of LiBr occurred within the first few hours. The rates of diffusion of tracer will be used to determine the appropriate type of diffusion model, and to distinguish diffusion from adsorption/desorption kinetics of NG and 2,4-DNT. These studies were supported by project number ER-1688 of the Strategic Environmental Research and Development Program (SERDP).

**TP100 A Kinetic Model for Munition Constituent Adsorption and Desorption** A. Miglino, R. Gonzalez, K. Michelson, H. Allen, D.M. Di Toro, Univ of Delaware, Dept of Civil Engineering. Partially detonated explosives are a source of concern on military ranges due to the potential for off-site transport. A kinetically based adsorption-desorption model for munition constituents (MCs) will be presented to predict behavior of explosive and propellant compounds. The model incorporates diffusive transport into particles where the MC can partition into organic matter. Calibration of the model reproduces adsorption data. Based on laboratory batch test data, a portion of the MC is resistive in desorbing from soil. This portion is not accurately described by only diffusion and partitioning. The results of a



two component reversible/resistant model will be presented to explain this behavior.

**TP101 Interference of Heavy Metals on Degradation of Nitroaromatic Compounds in Soil by Advanced Oxidation Processes** E. Jho, J. Jung, Seoul National Univ, Dept of Civil and Environmental Engineering; K. Nam, Seoul National Univ, Dept of Civil and Environmental Engineering, Seoul National Univ, Dept of Civil and Environmental Engineering. Many firing ranges in Korea are contaminated with both nitroaromatic compounds (NACs) such as trinitrotoluene and heavy metals such as lead (Pb) and copper (Cu). Feasibility of advanced oxidation processes (AOPs) for remediation of combined NACs and heavy metal contamination is studied by performing lab-scale experiments. Specifically, Fenton reactions are used to degrade dinitrotoluene (DNT) in soil in the presence and the absence of Pb and Cu. In addition, a range of hydrogen peroxide ( $H_2O_2$ ) concentrations will be investigated to study the effects of  $H_2O_2$  on DNT degradation in soils. The changes in the concentrations of DNT, Pb, and Cu in soil and in aqueous phase during Fenton reactions will contribute to identifying the effect of heavy metals on the DNT sorption in soil, hence, DNT degradation during Fenton reactions as sorbed organic compounds are not easily desorbed and degraded by Fenton reactions. We hypothesize that more DNT to be degraded by Fenton reactions in the absence of Pb or Cu as Pb or Cu compete with DNT for sorption sites on soil particles. Thus, in the presence of Pb or Cu, more DNT will be available in aqueous phase for degradation by radicals produced by Fenton reactions. The findings will contribute to the advancement of Fenton reactions for remediation of soils contaminated with NACs and heavy metals.

**TP102 Assessing Bio-uptake and Phytohydraulic Influence of Native Willow Trees in a Radioactively Contaminated Site** K.G. Keil, W.T. Frederick, M.R. Masser, S.P. Buechi, US Army Corps of Engineers, Buffalo District. During the 1940's, the Linde Corporation in Tonawanda, New York separated uranium ores to support the Manhattan Project. Radioactively contaminated materials from Linde were placed in the northern portion of the Tonawanda Landfill, a municipal landfill approximately 1.5 miles away. This area of the landfill remains uncapped, pending final assessment and potential remediation by the Federal Government. Samples of surface and subsurface soils, surface water, sediment, and groundwater, as well as from vegetative portions of a stand of willow trees growing within this uncapped portion of the landfill were used to assess the potential for human and ecological impacts from the localized subsurface radioactive contamination. The tree-focused samples (soil and water within the root zone; roots, trunk, and leaves) were intended to characterize potential impacts that the black willow trees have on groundwater flow through the landfill (phytohydraulics), and whether or not plant uptake of radionuclides was occurring. The soil samples taken within the shallow root zone of the trees had slightly elevated radioactivity, although less than nearby soils. Concentrations of radionuclides in vegetative samples were comparable to unimpacted soils. Unfiltered surface and groundwater samples obtained from within the tree root zone had concentrations of uranium (the most soluble and mobile of the radionuclides) greater than proximal groundwater samples. The groundwater levels and dissolved uranium concentrations in groundwater along the northern edge of the landfill show that the boundary of the landfill material with the natural lake-clay promotes an upward groundwater flow through the contaminated soil. Coincident groundwater and surface water impacts indicate that drainage features near the northern fence receive groundwater discharge containing uranium that was solubilized along the flow path through the radioactive material. This impacted groundwater then partially discharges to the surface water features and flows downgradient towards an off-site creek. These results indicate that while bio-uptake of radionuclides is not occurring into the black willow trees, the trees may be drawing in uranium-contaminated water toward their root zone, thereby affecting the uranium distribution in groundwater and receiving surface water discharges.

**TP103 Effects of Tungsten Chemical Species on Phosphate-dependent Pathways in a Bone Cell Line** D.R. Johnson, US Army Engineer Research and Development Center, Environmental Laboratory; C. Ang, Badger Testing Services; A.J. Kennedy, US Army Engineer Research and Development Center. Tungsten (W) is a metal that has numerous civil and military applications due to its high strength and melting point. When W enters the environment, it is rapidly oxidized and speciated based on the environmental matrix it is embedded in. Bone is the long-term storage organ

for W—and presumably W chemical species if present—when taken up by organisms. It is unknown what long-term effects W has in bone. Extensive polymerization of W to phosphate may deplete intracellular phosphate stores, disrupting phosphorylation reactions in cells. Furthermore, disruption of normal osteoblast function by W may impact bone formation (a phosphate-rich hydroxyapatite matrix) and biomechanics. Therefore, we evaluated the effects of W species on several phosphate-dependent intracellular functions, including energy cycling (ATP production), regulation of enzyme activity (protein tyrosine kinase [PTK]), and intracellular secondary messengers (cyclic adenosine monophosphate [cAMP]). hFOB 1.19 osteoblastic cells were exposed to vehicle control (water) or W chemical species (sodium tungstate, phosphotungstate [PW], poly tungstate [polyW], and tungstosilicic acid [TSA]) at 0-10,000  $\mu$ M for 24 h at 37 degrees C. Cells were then lysed and analyzed for cell viability, ATP concentration, tyrosine kinase activity, and cAMP concentration. W species did not affect ATP concentrations except at the highest concentration. PW, polyW, and TSA, but not tungstate, significantly increased protein tyrosine kinase activity at 10,000  $\mu$ M after 4 h exposure. Tungstate and PT increased cellular cAMP at  $\geq 100$   $\mu$ M, while TSA decreased cellular cAMP at 10,000  $\mu$ M. These data demonstrate that W chemical species generally only affect phosphate-dependent cell signaling and secondary messenger pathways in hFOB 1.19 cells. However, the W chemical species concentrations needed to affect these phosphate-dependent biochemical pathways greatly exceed the amount of W deposited in bone (100  $\mu$ M W = 18 mg/kg), thus providing evidence that W chemical species will not affect osteoblastic cellular activity.

**TP104 A Standardized Contact Transfer Method for Assessing Soil-to-Clothing Exposure to Chemical Agents** R.T. Checkai, The Ohio State Univ, School of Environment and Natural Resources, US Army Edgewood Chemical Biological Center, Environmental Toxicology, US Army Edgewood Chemical Biological Ctr, Dept of Environmental Toxicology; M.V. Haley, C.T. Phillips, M. Simini, US Army Edgewood Chemical Biological Center, Environmental Toxicology. Exposure to chemical warfare agents (CWA) can present a potential Contact Hazard even when the CWA compounds are sorbed onto soil. Previous studies have investigated levels of CWA transferred from contaminated surfaces utilizing a malleable latex material (dental dam; DD) as transfer substrate; however DD is typically inconsistent in compositions, and not a reliable simulant for toxicological investigations. Measurements of CWA on standard Army Combat Uniform (ACU), plus toxicological effects of CWA on mammalian skin, have been reliably established for some CWA, and ongoing-testing continues for others. But the need still existed for a reliable transfer-exposure method for assessing the exposure potential for CWA sorbed onto soil. We have established a method for reliably determining Contact Transfer of CWA compounds from soil directly onto clothing, utilizing standard mass (x Gravity) as a standard force to produce a standard measure of exposure potential. We initially determined the effects of contact time and applied mass (DD); then in benchmark investigations, we established the efficacy of CWA extraction from ACU (and DD), and Contact Transfer of CWA from soil onto clothing (ACU). A 4"-diameter circular swatch of ACU material was selected, similar to surface areas that may contact soil at knee or elbow locations; standard contact was created by placing the ACU swatch directly onto the soil-surface one minute after CWA dissemination, then covering the swatch with a 4"-diameter Plexiglass disk to distribute force from a centrally-placed standard mass. Contact Transfer of CWA was determined by solvent extraction of swatches, with subsequent analyses by GC/GC-MS. Masses 0.250, 0.500, 1.000, 1.500 kg, resulted in significantly greater CWA transfer ( $p \leq 0.05$ ) at  $\geq 0.500$ kg compared to 0.250kg; therefore the 1-kg standard mass was selected for integration into the method. Although the quantities of soil-sorbed CWA that transferred increased as a function of contact time, the rate of transfer decreased dramatically after 1h; therefore 0.25h was selected for integration into the method. The quantities of chemical agents transferred from soil surfaces contaminated at operationally important levels from Soil-to-Clothing (ACU) are amounts that are within the range of concern for Soldiers. The standardized Contact Transfer Method provides reliable standard predictions of exposure potential, and in conjunction with toxicity data for predictions of Contact Hazard.

**TP105 A Study of Mercury and Neurochemical Biomarkers Across Several Fish Species** D. Nam, Univ of Michigan, Dept of Environmental Health Sciences; D. Adams, Fish & Wildlife Research Institute; D. Evers, Biodiversity Research Institute; J. Head, Univ of Michigan, Cooperative

Institute of Limnology and Ecosystem Research, Univ of Michigan, Cooperative Institute for Limnology and Ecosystem Research; M.J. Carvan, Univ of Wisconsin-Milwaukee, School of Freshwater Sciences, Univ of Wisconsin-Milwaukee, Childrens Environmental Health Sciences Center; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. Mercury (Hg) is a potent neurotoxicant that impairs key physiological processes related to vertebrate behavior and reproduction. Recent studies in fish have also established that Hg is a neuroendocrine disruptor, though little is known about the sub-clinical effects and the underlying mechanisms. Here we present findings from a series of studies (in vitro, field work, laboratory bioassay) that aimed to assess Hg-associated neurochemical effects in several fish species as follows: 1) studies on wild fish (lemon shark, mako shark, seatrout, largemouth bass); 2) in vitro screening assays (lemon shark, mako shark, yellow perch, goldfish) on neurochemical receptors and enzymes underlying vertebrate reproduction; 3) studies on laboratory-exposed yellow perch. In the wild fish studies, mean brain Hg levels were: 0.145 ppm dry wt in lemon shark (n=28; South Florida), 1.69 ppm in mako shark (n=12; Atlantic coast), 0.577 ppm in seatrout (n=28; South Florida), and 0.069 ppm in South River fish (n=28; Virginia). Saturation binding curves were developed from each fish species to obtain Bmax (195 – 266 fmol/mg for mAChR, 236 – 3065 fmol/mg for NMDAR) and Kd (3.18 – 4.64 nM for mAChR, 28.9 – 368 nM for NMDAR). Based on inhibition constants (Ki) against Hg<sup>2+</sup> and CH<sub>3</sub>Hg<sup>+</sup>, goldfish and yellow perch were the most sensitive to mAChR and NMDAR binding, respectively. When brain Hg was related to neurochemical enzymes (MAO, AChE) and receptors (mACh, NMDA), there were no Hg-associated neurochemical changes except in the mako shark where a negative correlation with NMDA glutamate receptors ( $r = 0.496$ ;  $p < 0.05$ ) was found. Mercury inhibited glutamate uptake in synaptosomes from seatrout and two shark species in a dose-dependent manner (IC<sub>50</sub>: 0.39–2.3  $\mu$ M of Hg<sup>2+</sup>; 0.15–2.1  $\mu$ M of CH<sub>3</sub>Hg<sup>+</sup>). Via cell-free in vitro screening assays, we are assessing Hg-related impacts towards sex hormone receptors, neurotransmitter release and uptake mechanisms, and neurochemical receptors and enzymes that have critical roles in reproduction (e.g., dopamine and GABA systems) and neurobehavior (e.g., glutamate pathway). We will also report on neurochemical data from laboratory-exposed perch.

**TP106 Comparing the Sensitivity of Wild Populations of *Carcinus maenas* from Two Estuaries of the NW Coast of Portugal to the Insecticide Fenitrothion**

A. Rodrigues, Univ of Porto – ICBAS & CIIMAR, ICBAS – Institute of Biomedical Sciences of Abel Salazar, Dept of Populations' Studies, Porto, Portugal; CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, Porto, Portugal.; P. Oliveira, Univ of Porto, CIIMAR, CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, Laboratory of Ecotoxicology and Ecology, Porto, Portugal.; L. Guilhermino, Univ of Porto, CIIMAR & ICBAS, Laboratory of Ecotoxicology, Univ of Porto, ICBAS & CIIMAR, Universidade do Porto; L. Guimaraes, Univ of Porto, CIIMAR & ICBAS. Sensitivity to fenitrothion of crab populations from the NW Portuguese estuaries of rivers Minho and Lima was assessed through laboratory exposure bioassays. Minho is a low impacted estuary, which has been used as reference in previous studies. Lima estuary receives inputs from urban and industrial sources, it has a harbour and a shipyard at its mouth, and field crops are located in its vicinity. Fenitrothion was selected as test substance because it is a widely used organophosphate insecticide (OP), with a well known mechanism of primary toxicity (acetylcholinesterase inhibition). In two bioassays running in parallel, crabs from the two populations were individually exposed to several concentrations of fenitrothion. In each bioassay, 10 crabs were used per treatment. Mortality was recorded at each 24h. After 7 days of exposure, surviving animals were sacrificed, the activity of target enzyme was determined and mechanisms of biotransformation and defence against toxicant insults were investigated. Overall, the results indicate that the population of Lima estuary is less sensitive to fenitrothion than the population of the Minho estuary, suggesting that crabs developing in the most contaminated estuary may have developed tolerance to pollution. This work was supported by funds from the Portuguese Foundation for the Science and Technology (FCT) and EU-FEDER funds through the Programme COMPETE (Project CRABTHEMES FCOMP-01-0124-FEDER-007383). A.R. was supported by a PhD training grant from FCT (SFRH/BD/65456/2009).

**TP107 Developmental Bisphenol A Exposure Results in Abnormal Larval and Adult Zebrafish Behavior** D. Weber, Univ of Wisconsin-Milwaukee, Childrens Environmental Health Sciences Center, Univ of Wisconsin-Milwaukee, Great Lakes Water Institute, Univ of Wisconsin-Milwaukee; A. Patel, Univ of Wisconsin-Milwaukee, Dept of Psychology; K.S. Saili, Oregon State Univ, Environmental & Molecular Toxicology, Oregon State Univ, Environmental & Molecular Toxicology; M.M. Corvi, S.R. Das, R. Tanguay, Oregon State Univ, Environmental & Molecular Toxicology. Bisphenol A (BPA) is a high production volume chemical found in polycarbonate plastic, resin can linings, and thermal printing paper. Ingestion is considered the primary route of human exposure. BPA is a suspected estrogen disruptor that may interfere with central nervous system (CNS) development. To determine whether environmentally relevant levels of BPA impact CNS development, we assessed the effect of BPA on larval and adult behavior endpoints. For the larval behavior tests, developing zebrafish embryos were exposed to a range of nonteratogenic BPA concentrations (0.001 to 10  $\mu$ M) during neurogenesis (10 – 58 hours post fertilization, hpf). The 0.01, 0.1, and 1  $\mu$ M exposure concentrations resulted in behavioral hyperactivity (response to light on-light off) in 120 hpf larvae (N=32-64/concentration; one way ANOVA,  $P < 0.05$ ). For the adult behavior tests, zebrafish embryos were exposed as above to 0.1 or 1  $\mu$ M BPA. Both exposure concentrations resulted in abnormalities in adult learning behavior. Cognitive flexibility, a measure of executive function, was examined in a computer-controlled reversal task. Individual adult fish (N=10; 8 months old) freely chose the left or right arm of a T-maze. Initial choice resulted in a mild shock. Criterion achieved upon 3 consecutive choices of non-shocking arm at which time an additional choice of that arm resulted in a shock. Criterion for second reversal achieved with 3 consecutive choices of newly, computer-designated non-shocking arm. While no significant differences were observed between male and female fish, there were significant, concentration-dependent differences in trials to first reversal, trials to second reversal, consecutive incorrect trials to first reversal, consecutive incorrect trials to second reversal, and number of larvae completing each reversal (one way ANOVA,  $P < 0.005$ ). Research was supported by NIEHS T32ES7060, ES00210, ES04184, and EPA STAR Graduate Fellowship (KSS).

**TP108 Effects of Methylmercury Chloride on Neurochemical Biomarkers in the Developing Chicken Embryo** J. Rutkiewicz, Univ of Michigan;

D. Nam, Univ of Michigan, Dept of Environmental Health Sciences; J. Head, Univ of Michigan, Cooperative Institute of Limnology and Ecosystem Research, Univ of Michigan, Cooperative Institute for Limnology and Ecosystem Research; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. Mercury (Hg) is an established neurotoxicant that has been linked to neurobehavioral changes in birds in both field and laboratory studies. Recently, changes in receptors and enzymes involved in neurotransmission have been studied as subclinical biomarkers of Hg's neurological effects in wildlife, including birds. These changes precede structural changes and potentially warn of changes in ecologically relevant behaviors. Avian embryos are exposed to maternally deposited methylmercury (MeHg) throughout development, but its effects on the embryonic brain are relatively unexplored. The objective of this work is to determine if in ovo MeHg exposure in chicken embryos is associated with alterations in the NMDA and GABA receptors and glutamine synthetase (GS) and glutamic acid decarboxylase (GAD) enzymes, which have previously been studied as biomarkers in wild bald eagles. White leghorn chicken eggs were injected with MeHg chloride (Study 1: 0, 0.17, 0.62, or 2.0 mg/g egg; Study 2: 0, 0.62, 2, 3.2, or 6.2 mg/g egg) and were incubated until day 19, upon which embryos were dissected and brains (dissected into cerebrum, cerebellum, and optic lobe regions) were frozen until analysis. In both studies, mortality ranged from 0% to 29% and did not increase in a dose dependent manner. Brain Hg concentrations increased with dose in both studies, reaching a high mean concentration of 0.85 mg/g in the highest dose group in study 1 and 2.2 mg/g in the highest dose group in study 2. In study 1, Hg was not associated with receptor binding or enzyme activity in any brain region. Despite the higher brain Hg concentrations observed in study 2, no associations were found with binding to the NMDA receptor or GABA receptor in any brain region. Effects on GS and GAD activity are currently being studied. These studies suggest that unlike in adult birds, MeHg associated changes to the aforementioned neurochemical biomarkers may not occur in late stage embryos. Upcoming studies will investigate the effects of Hg exposure on neurochemistry at earlier developmental stages to determine if Hg disrupts critical developmental processes



in the brain, continue to study other neurochemical systems, and explore linkages between exposure and chick neurobehavior.

**TP109 Effects of Non-selective Cyclooxygenase Inhibitor, Ibuprofen, on Gene Expression, Prostaglandin Synthesis and Reproductive Behavior in Zebrafish**

D. Martinovic-Weigelt, Univ of St. Thomas, Dept of Biology, USEPA, Mid-Continent Ecology Division, Mid-Continent Ecology Division, Univ of St. Thomas, Biology; J. Marchuk, Univ of St. Thomas, Dept of Biology; T. Firkus, Univ of St. Thomas. Prostaglandins are a class of hormones important for the regulation of gonadal steroidogenesis and ovulation in vertebrates. In fish they are also used as pheromones; they are released by ovulating females and are important for the initiation and synchronization of male reproductive behaviors. Commonly used pharmaceuticals such as nonsteroidal anti-inflammatory pharmaceuticals (e. g. ibuprofen (IB) etc.) inhibit cyclooxygenase (COX) enzymes which catalyze prostaglandin synthesis. Sexually mature zebrafish males (n=14) and females (n=14) were exposed to 50 µg/L of IB for 14 days and their reproductive behaviors were compared to well-water exposed controls (n=14 per sex). The present study also examined effects of IB exposure on prostaglandin synthesis pathway; we measured phospholipase, COX 1 and 2, and prostaglandin synthase mRNA abundance using real time polymerase chain reaction. In addition, the ovarian prostaglandin F2 alpha concentrations and COX enzyme activity, were evaluated. Exposure to IB caused decrease in COX activity and was mirrored downstream in the reduced (but not significantly different) production of PGF-2α. Our lack of ability to detect effects on PGF-2α was likely, in part, a result of a high variability due to the asynchronous ovarian maturation. Furthermore, exposed fish may have been able to compensate for the decreased COX activity by increasing abundance of mRNA for COX and prostaglandin synthases (as demonstrated by our gene expression data). Several courtship behaviors were significantly decreased in IB-exposed males, but they were not altered in females. These findings suggest that IB affects prostaglandin synthesis pathway and impairs performance of male sexual behavior and thus has a potential to impair the reproductive success of exposed individuals.

**TP110 Effects of Non-selective Cyclooxygenase Inhibitor, Ibuprofen, on Gene Expression, Prostaglandin Synthesis and Reproductive Behavior in Zebrafish**

T. Firkus, Univ of St. Thomas; D. Martinovic-Weigelt, Univ of St. Thomas, Dept of Biology, USEPA, Mid-Continent Ecology Division, Mid-Continent Ecology Division, Univ of St. Thomas, Biology; J. Marchuk, Univ of St. Thomas. Prostaglandins are a class of hormones important for the regulation of gonadal steroidogenesis and ovulation in vertebrates. In fish they are also used as pheromones; they are released by ovulating females and are important for the initiation and synchronization of male reproductive behaviors. Commonly used pharmaceuticals such as nonsteroidal anti-inflammatory pharmaceuticals (e. g. ibuprofen (IB) etc.) inhibit cyclooxygenase (COX) enzymes which catalyze prostaglandin synthesis. Sexually mature zebrafish males (n=14) and females (n=14) were exposed to 50 µg/L of IB for 14 days and their behaviors (agonistic, courtship, and swimming behaviors) were compared to well-water exposed controls (n=14 per sex). The present study also examined effects of IB exposure on prostaglandin synthesis pathway; we measured phospholipase, COX 1 and 2, and prostaglandin synthase mRNA abundance using real time polymerase chain reaction. In addition, the ovarian prostaglandin F2 alpha concentrations and COX enzyme activity, were evaluated. Exposure to IB caused decrease in COX activity and was mirrored downstream in the reduced (but not significantly different) production of PGF-2 alpha. Our lack of ability to detect effects on PGF-2 alpha was likely, in part, a result of a high variability due to the asynchronous ovarian maturation. Furthermore, exposed fish may have been able to compensate for the decreased COX activity by increasing abundance of mRNA for COX and prostaglandin synthases (as demonstrated by our gene expression data). Several behaviors (agonistic, courtship and general activity) were significantly decreased in IB-exposed males, but they were not altered in females. These findings demonstrate that IB affects prostaglandin synthesis pathway, and impairs performance of fish reproductive behaviors. In conclusion, exposure to wastewater effluents (which frequently contain IB and other cyclooxygenase inhibitors) may impair reproductive success of fish.

**TP111 Embryonic Exposure to Deltamethrin Results in Dopaminergic Gene Expression Changes and Behavioral Alterations in the Zebrafish**

T.S. Kung, Rutgers, the State Univ of New Jersey; J.R. Richardson, Univ

of Medicine and Dentistry, New Jersey; K.R. Cooper, Rutgers, the State Univ of New Jersey, Dept of Biochemistry and Microbiology; L.A. White, Rutgers, the State Univ of New Jersey. Pyrethroids are one of the most commonly used insecticides and, when used properly, are generally considered to pose little risk to human and environmental health. However, there has been increasing concern that children are more susceptible to the adverse effects of pesticides. Previous work from our laboratory characterized the acute developmental toxicity of six common pyrethroids in the zebrafish and it was determined that the toxicities associated with acute exposures are similar to what has been shown in mammalian models, thus validating the zebrafish model system. Here, we are using the zebrafish model to test the hypothesis that developmental exposure to low doses of pyrethroid pesticides alters dopaminergic neuronal development, resulting in long-term gene expression changes and behavioral alterations. To assess the effects of low dose pyrethroid exposure during development, we exposed zebrafish embryos to deltamethrin at a dose below the LOAEL (0.5 mg/L), during the embryonic period (3-72 hpf) using a static non-renewal water exposure. We found that embryos exposed to deltamethrin exhibited dopaminergic gene expression changes at 72 hpf, including increased expression of the dopamine transporter (DAT) (1.6-fold) and the dopamine receptor D1 (DRD1) (2.1-fold), which persists for at least 1-month post-exposure. To determine whether developmental deltamethrin exposure altered swimming behavior, we quantified the swim behavior of 6-week old zebrafish using the Noldus Ethovision System and found that treated zebrafish exhibited statistically significant increases in swim activity (3-fold) as measured by the total distance traveled in 30 min. These fish at 6 months of age also exhibited statistically significant increases in aggressive behavior; quantified by counting the number of times they attacked their own image in a mirror. Interestingly, this increase in aggression observed only in the male fish. Fish exhibiting altered behavior and the respective control fish were grown to sexual maturity and bred to obtain F<sub>1</sub> generation fish. The F<sub>1</sub> generation fish exhibited decreased expression of DAT (1.6-fold) and DRD1 (2.6-fold) and increased swim activity, suggesting that these changes may persist into the next generation. Our data indicate that developmental exposure to deltamethrin results in persistent dopaminergic gene expression changes and behavioral deficits. Funded by NIEHS ARRA432298, ES005022, and ES015991

**TP112 Environmentally Relevant Concentrations of the Flame Retardant BDE-209 in Sediment Linked to Potential Developmental Effects on Zebrafish Embryos**

N. Garcia-Reyero, Jackson State Univ; L. Escalon, US Army, Engineer Research & Development Center; E. Prats, CSIC; B. Thienpont, IDAEA-CSIC; P. Babin, Universités Bordeaux 1 et 2; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division; D. Raldua, IDAEA-CSIC. Decabromodiphenyl ether (BDE-209) is a fully brominated diphenyl ether compound used as a flame retardant in polystyrene applications such as casings for computers, upholstery textile and televisions. It is highly lipophilic and persistent and as such it is prone to bioaccumulation and biomagnifications in the food chain. Increasing concentrations of polybrominated diphenyl ethers in wildlife have been documented since the mid 1990s. BDE-209 is a very large molecule, and the general thought was that due to its size it was not bioavailable, and therefore could not bioaccumulate in wildlife. Several studies now have proven this concept wrong, as BDE-209 has been found in many aquatic and terrestrial organisms. Concentrations as high as 707 ng BDE-209/g lipid weight have been found in carp (*Cyprinus carpio*) and barbel (*Barbus graellsii*) and values as high as 12 µg/g dry have been found in sediments downstream of an industrial park in Spain. Furthermore, concentrations as high as 1.23 µg/g lipid have been found in several fish species in Lake Michigan (USA). BDE-209 has been linked to decreased otolith increment widths in whitefish as well as failed eggs from white stork (*Ciconia ciconia*) in Spain. Exposure of Sprague-Dawley rats to BDE-209 resulted in significantly increased liver, thyroid, and adrenal gland weights, in addition to a reduction in total serum triiodothyronine (T<sub>3</sub>) and increase of thyroid-stimulating hormone levels. Exposure to BDE-209 has also been linked to decreased learning and memory in mice, behavioral changes in mice and inhibition of neural stem cells into neurons in culture. As many of the toxic effects of BDE-209 have been found on laboratory exposures, here we analyze the potential effects of an environmentally relevant dose of BDE-209 on zebrafish (*Danio rerio*) embryo. Zebrafish embryos were exposed to sediments that contained 12,500 mg/kg BDE-209 for eight days. We analyzed gene expression changes, thyroid function, and morphometric



parameters. While we did not detect changes in thyroid function, gene expression analysis highlighted the potential effects of BDE-209 on development, neurotoxicity, and liver toxicity.

**TP113 Using Zebrafish Model (*Danio rerio*) to Investigate the Effects of Pyrethroid Insecticide Deltamethrin on Glutamatergic System** A. Truong, Rutgers, the State Univ of New Jersey, Dept of Biochemistry and Microbiology; T.S. Kung, Rutgers, the State Univ of New Jersey; K.R. Cooper, L.A. White, Rutgers, the State Univ of New Jersey, Dept of Biochemistry and Microbiology. Deltamethrin is a chemical belonging to the pyrethroid insecticide family derived from the natural compound found in the plant species *Chrysanthemum cinerariaefolium*. These insecticides are modified from their parent compound so that they are more potent to insects and more resistant to degradation. After the banning of organophosphates in the United States, this class of insecticides became very popular in household use. In the frequent testing conducted by the NHANES, the metabolite 3-phenoxybenzoic acid of pyrethroids was found in urine samples of children. These children also showed signs of hyperactivity. This study showed that children are being exposed to the compounds. In an effort to understand the effects of pyrethroid insecticides at doses below the lowest observed adverse effect level, several laboratories have found the changes in DAT expression level and as well as other genes involved in the dopaminergic system using mice and zebrafish. Results from these studies correspond to the previously observed relationship between change in dopaminergic system and hyperactive behavior. Some studies also showed the connections between the dopaminergic system and several other systems such as the glutamatergic. In this study we hypothesized that developmental deltamethrin insecticide exposure results in changes in the glutamatergic gene expression.

**TP114 Use of Macrophage Immunoassays to Assess the Toxicity of Different OSPW Samples** J. Ge, E. Garcia-Garcia, A. Oladiran, Univ of Alberta, Dept of Biological Sciences; M. Gamal El Din, L.C. Perez-Estrada, J.W. Martin, Univ of Alberta, Dept of Civil and Environmental Engineering; M. Belosevic, Univ of Alberta, Dept of Biological Sciences. The Oil Sands Process Water (OSPW) produced by the mining industry in northern Alberta constitutes a major environmental challenge. Naphthenic acids (NAs) are thought to be the primary toxic component in OSPW. Different OSPW samples are known to possess particular NAs compositions. It is unclear whether different NAs contents cause different toxic effects in multicellular organisms. In this study, we used in vitro bone marrow-derived macrophage (BMDM) assays to assess the toxicity of the organic fractions of two OSPW samples (OSPW1 and OSPW2) that have different NAs content. The extracted organic fraction of OSPW1 constituted 43% NAs, while that of OSPW2 was only 29%. OSPW2 had more NAs of higher molecular weight (carbon number) and ring number (Z values) than OSPW1. BMDM were exposed to increasing concentrations of NAs present in the extracted organic fraction of the OSPW samples, and the expression of pro-inflammatory genes, IL-1, IL-6, IL-12 and TNF- $\alpha$ , was determined using quantitative real-time PCR (qPCR). Both OSPW samples reduced non-stimulated expression of IL-1 and IL-12. OSPW2 also inhibited non-stimulated expression of IL-6, and increased the expression of TNF- $\alpha$ . Both OSPW samples decreased the LPS+IFN $\gamma$ -stimulated expression of IL-1, but in addition OSPW2 increased the LPS+IFN $\gamma$ -stimulated expression of IL-12 and TNF- $\alpha$ . Phagocytosis of BMDM was significantly reduced in both OSPW1 and OSPW2 treated cells. Both OSPW samples reduced the respiratory burst induced by PMA stimulation. OSPW1 significantly reduced the expression of the NADPH oxidase subunit p91<sup>phox</sup>, while OSPW2 reduced the expression of the p47<sup>phox</sup> and p67<sup>phox</sup> subunits. Both OSPW samples reduced the production of nitric oxide, in activated BMDM, and the decreased nitric oxide production was related with reduced expression of the iNOS gene. Our results indicate that different NAs compositions in OSPW can have different effects on BMDM antimicrobial responses.

**TP115 Mast Cell Function is Agonized by Exposure to Environmental Estrogen 4-Tert-Octylphenol** R.K. Palmer, Univ of Maine, Molecular and Biomedical Sciences, The Univ of Maine, Molecular and Biomedical Sciences; J.H. Pelletier, Univ of Maine, Molecular and Biomedical Sciences; J.A. Gosse, Univ of Maine, Molecular and Biomedical Sciences, Univ of Maine, Graduate School of Biomedical Sciences. In addition to common industrial and pesticide applications, surfactants (wetting agents) are widely used in personal care products (e.g., soap and shampoo), and are also employed after oil spills as dispersants. Alkylphenol polyethoxylates (APEOs) are a major

class of non-ionic surfactants that are known to pollute rivers, surface water, and tap water after their intended application. Stable metabolites of APEOs, such as nonylphenol (NP) and 4-*tert*-octylphenol (OP), have demonstrated estrogenic endocrine disrupting ability both in vitro and in vivo, which extends great concern, as they are found at detectable levels in biological samples of aquatic and terrestrial species. Adverse health impacts are known to arise from exposures to environmental endocrine disrupting chemicals, but not much is known about the ways in which they interfere with normal immune function and host defense mechanisms. Although a number of immunotoxicological effects due to sex steroids have been described (Watson & Gametchu, 2001, *Int. Immunopharm.*), very few have been explored mechanistically. Mast cells are immune effector cells that participate in both inflammatory and allergic signaling pathways, and are also the key players in the defense against parasitic attack. Recently, it was demonstrated in human and murine mast cells that estrogen receptor agonists amplify the degranulation function of these cells, resulting in elevated release of preformed allergic mediators from cytosolic granules (Narita, et al, 2007 *Environ. Health Persp.*; Zaitse, et al., 2007 *Molec. Immunol.*). Using a fluorescent microplate assay to quantify the release of  $\beta$ -hexosaminidase from estrogen-starved mast cells (RBL-2H3), we demonstrate that OP amplifies IgE-dependent mast cell degranulation in a dose-dependent fashion following 1-h exposure: by 10%  $\pm$  2% at 5  $\mu$ M, 22%  $\pm$  2% at 10  $\mu$ M, and 34%  $\pm$  7% at 20  $\mu$ M (mean  $\pm$  SEM). Interestingly, we found these effects to require a co-stimulatory agent (i.e., multivalent antigen), as exposure to OP, alone, did not alter the degranulation endpoint. Importantly, doses used in the degranulation assay were found non-cytotoxic in lactate dehydrogenase and clonogenic cytotoxicity assays. We anticipate that these immunological effects are dependent on Estrogen Receptor (ER)- $\alpha$ , and in future studies will use an anti-estrogen to pharmacologically inhibit this receptor on the surface of mast cells.

**TP116 An Investigation of the Effects of Bisphenol-A on the Development and Differentiation of Embryonic C57BL/6 Mice Thymocytes** L. Leung Liu, The Univ of La Verne, Univ of La Verne; C. Broussard, The Univ of La Verne. The immune system is composed of structures and processes that help defend and protect organisms from pathogens and cancer. However, exposure to endocrine disrupting chemicals (EDCs) can disrupt the normal function of the immune system due to the capability of EDCs to mimic or block natural hormones. These effects can alter proper immune function and can lead to an increase in susceptibility to infectious diseases and an increase in autoimmune diseases. Great concern has arisen regarding an endocrine disruptor called bisphenol-A (BPA) that is used as an additive for the production of polycarbonate plastic and epoxy resin. Numerous studies have been performed to look at the effects of BPA in the reproductive system and in the nervous system; however, few studies have looked at the effects on the developing embryo, in particular on embryonic T cell development. The aim of this study was to investigate the effects of BPA at the gestational development stage on the different subpopulations of embryonic T cells. An in vitro assay was used to examine the development of T cells at different concentrations of BPA (0, 6.25, 12.5, 25, 50, and 100  $\mu$ M). T cells were extracted from C57BL/6 mice embryos at 16 to 18 days of gestation. Our results indicate a dose dependent decrease in viability of the total number of T cells, as well as in the different T cell subpopulations. It was also found that the decrease in T cell viability was significant at 100  $\mu$ M. These findings suggest that BPA affects normal development and survival rate of embryonic T cells, which can have detrimental effects on the organism's immune system. This research is supported by the Univ of La Verne Faculty Research Committee. The opinions expressed in this work are solely the authors'.

**TP117 Estrogen Receptor Usage by Endocrine Disrupting Chemical DES and HPTE in Embryonic Thymocyte Differentiation** A. Lim, Univ of La Verne; C. Broussard, The Univ of La Verne, Univ of La Verne, Biology, Univ of La Verne, Professor of Biology. Endocrine disrupting chemicals (EDCs) are synthetic organic compounds such as additives in plastic and pesticides that are commonly found in the environment. Research shows that EDCs are estrogen mimicking chemicals that can bind to estrogen receptors on a cell, disrupting normal hormonal actions. In addition, they can mimic or block endogenous hormones by behaving as biological signals and, thus, can be easily misinterpreted by an organism's cell receptors. Evidence has shown that EDCs negatively affect the reproductive fitness of adults and the developing endocrine system of embryos. EDCs can also increase the risk of cancer in adults and increase the frequency of allergic diseases and

asthma in children. EDCs are especially detrimental to developing embryos because early lifetime exposures, such as during fetal or early postnatal periods, produce permanent effects. Two known EDCs that have been extensively studied in the past are diethylstilbestrol (DES) and 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE). Research shows that both DES and HPTE suppress the development of fetal immune systems with their estrogenic properties. However, little is known about the pathways that these two chemicals utilize to mediate their effects on the immune system of the body. There is no conclusive research on which estrogen receptors they bind to on the surface of T-cells in order to exert their effects on the cells. The focus of this study will be to determine which receptors DES and HPTE bind to on the surface of T-cells – whether they are the classical estrogen receptors, ER alpha and ER beta, or the nonclassical estrogen receptor, GPR 30. By gaining a better understanding of how EDCs interact with T-cells, we will be able to better understand the effects of EDCs on the immune system.

**TP118 Per- and Poly-fluorinated Compound Occurrence, Profiles, and Time Trends in Landfill Leachate** J.P. Benskin, AXYS Analytical Services Ltd; B. Li, Univ of British Columbia, Civil Engineering; M. Ikonou, Fisheries and Oceans Canada, Contaminants Science Section; J. Grace, Univ of British Columbia, Chemical and Biological Engineering; L. Li, Univ of British Columbia, Civil Engineering. Per- and poly-fluorinated compounds (PFCs) have been used ubiquitously in consumer and commercial products for over 60 years. Many of these products are eventually disposed of in landfills, where PFCs may leach from them over time. In this study, we investigate the occurrence, profiles, and trends of PFC and PFC-precursors in landfill leachate, collected from February to June 2010 from a municipal landfill. Perfluorohexanoate (PFHxA) and perfluorooctane sulfonate (PFOS) were typically the dominant PFCs (present at up to several ppb); however, significant changes in the relative congener profile were observed over the 5-month sampling campaign. Most notably, PFOS concentrations increased by an order of magnitude from mid-March to mid-April, and then returned to original levels. This increase was strongly correlated to an increase in PFOS-precursor concentrations (e.g., perfluorooctane sulfonamide and N-alkyl substituted perfluorooctane sulfonamido acids) over the same time period. A similar correlation was observed between several perfluorocarboxylates (PFCAs), and PFCA-precursors (e.g., 8:2 and 10:2 fluorotelomer carboxylates (PFCAs), and PFCA-precursors (e.g., 8:2 and 10:2 fluorotelomer acids), while for other congeners (e.g., perfluorohexane sulfonate and PFHxA), no increase was observed. Experiments were performed to investigate potential causes for the increase in PFC and PFC-precursor concentrations. One possibility is increased (bio-) degradation of higher precursors incorporated into various commercial and consumer products. These include phased-out materials such as the sulfonamide-linked phosphate diesters and fluoroacrylate polymers, or analogous telomer-linked polymers and surfactants manufactured today. Examination of PFOA isomer profiles suggests that contributions from both historical and contemporary production are significant. Overall, these data indicate that PFC-containing products disposed of in landfills may be a long-term source of PFCs. Management of landfill leachate through containment, collection, and treatment is important to prevent potential impacts to the environment.

**TP119 Ultra-fast Separation of PFOS and PFOA Isomers** J.P. Benskin, AXYS Analytical Services Ltd; M. Ikonou, Fisheries and Oceans Canada, Contaminant Sciences Division; M.B. Woudneh, AXYS Analytical Services Ltd, R&D Chemist; F.A. Gobas, Simon Fraser Univ, School of Resource and Environmental Management (Faculty of Environment), Simon Fraser Univ; J.R. Cosgrove, AXYS Analytical Services Ltd. Historical manufacturing of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) by Simmons electrochemical fluorination results in an impure product of 70-80% linear and 20-30% branched isomers. Recent data demonstrated that branched perfluorinated acid (PFA) isomers can display significantly different physical, chemical, and biological properties, compared to their linear counterparts. Branched isomers can also respond differently to electrospray ionization and act as a potential source of error in total PFA quantification if they are not considered separately. Despite this, the most common HPLC-MS/MS methods for quantifying PFOS and PFOA involve eluting all isomers together and integrating them as a single peak. In part, this is because few methods currently exist for separation of branched from linear PFAs, and those that are in use employ long instrument runs (i.e., >30 min) or suffer from poor column stability. Here, we present an ultra fast (< 15 min) HPLC-MS/MS method for separation of PFOS and PFOA isomers

using a fused-core perfluorophenyl stationary phase. Fused core HPLC-columns facilitate the use of high flow rates without a concomitant increase in back pressure. For PFOA, a total of 4 isomer peaks could be resolved to baseline in the m/z 413/369 product ion, while for PFOS, linear, total monoperfluoromethyl, and total diperfluoromethyl isomers were resolved to baseline in the m/z 499/80 product ion. Using characterized technical standards, branched and linear PFOS and PFOA isomers were quantified separately. This method provides potentially improvements in analytical accuracy and data quality over traditional methods, without the corresponding increase in costs or analysis time associated with previous isomer separation methods.

**TP120 Gas/Particle Partitioning of Perfluoroalkyl and Polyfluoroalkyl Substances in the Atmosphere Using High Volume and Diffusion Denuder Samplers** L. Ahrens, Environment Canada, Science and Technology Branch; M. Shoeib, Environment Canada, Atmospheric Science and Technology Directorate; D.A. Lane, Environment Canada; T. Harner, Environment Canada, Atmospheric Science and Technology Directorate; R. Guo, Univ of Toronto; R.J. Reiner, Ontario Ministry of the Environment. Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are globally distributed in the environment by atmospheric and oceanic transport. Gas/particle partitioning is a key determinant of environmental fate and long-range transport (LRT) potential of PFAS. Previous studies using conventional high volume air samplers (hi-vol) utilizing glass-fiber filters (GFFs) for capturing the particle phase have shown that the majority of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs) are present in the particulate phase. However, sampling artifacts were reported for perfluorooctanoic acid (PFOA) showing that vapour phase PFOA can “blow-on” the GFF and can adsorb to particulate matter already collected on the GFF, resulting in an overestimation of the particle phase concentration. In this study, PFAS were determined in the gas and particulate phase using co-located hi-vol and diffusion denuder samplers at an urban site in Toronto, Canada, from November to December 2010. Diffusion denuders overcome the ‘blow-on’ artefact because the gas-phase is collected first (using highly sorptive ground XAD-4 coated onto the annular denuder surface) followed by the particle-phase. Samples were analyzed for 7 PFAS classes (i.e., fluorotelomer alcohols (FTOHs), fluorotelomer acrylates (FTACs), fluorotelomer methacrylates (FTMACs), perfluorooctane sulfonamides (FOSAs), perfluorooctane sulfonamidoethanols (FOSEs), PFCAs and PFSAs). Total air concentrations for PFAS measured using hi-vol and diffusion denuder samplers were within a factor of two, however, much greater differences were observed for gas/particle partitioning. Hi-vol measurements resulted in much higher particle-associated fractions for perfluorooctane sulfonic acid (PFOS) and PFCAs compared to the diffusion denuder sampler. For example, PFOA had a particle-associated fraction of ~70% using hi-vol samplers whereas only ~3% was associated with particles using the annular diffusion denuder samplers. These results highlight problems associated with using conventional hi-vol samplers for assessing gas/particle partitioning of some PFASs due to sorption of gas-phase chemical onto the GFF. Overall, the results of this study improve our understanding of the gas/particle partitioning of PFASs in the atmosphere which is important for global transport models used to assess the LRT potential of PFASs.

**TP121 Effect of Perfluorinated Compounds (PFCs) on Maternal Thyroid Hormones During Early Pregnancy: Results from the CHirP Study (Vancouver, Canada)** G.M. Webster, Univ of British Columbia, School of Population and Public Health, School of Environmental Health, Univ of British Columbia; S.A. Venners, Simon Fraser Univ, Faculty of Health Sciences; A. Mattman, St Paul's Hospital, Pathology and Laboratory Medicine; J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine and Pathology. Perfluorinated compounds (PFCs) are used as stain, grease and water repellents in many consumer applications and are detectable in most human serum samples. Although PFCs are known thyroid toxicants in animal studies, very little is known about their potential to disrupt thyroid hormone levels in humans. Thyroid disruption during early pregnancy is of particular concern, as maternal thyroid hormones in early gestation play a critical role in fetal brain development. This study examined the associations between perfluorohexanesulfonate (PFHxS), perfluorononanoate (PFNA), perfluorooctanoate (PFOA), perfluorooctanesulfonate (PFOS), sum perfluorosulfonates (SumPFSAs), sum perfluorocarboxylates (SumPFCAs), and sum perfluorinated compounds (SumPFCs) and free thyroxine (fT4), total thyroxine (TT4) and thyroid stimulating hormone (TSH) in



152 euthyroid pregnant women enrolled in the Vancouver (Canada) – based Chemicals, Health and Pregnancy study (CHiP). Mixed models were used to examine associations between PFCs (measured in maternal serum at 15 weeks gestation) and thyroid hormones (measured in maternal serum at 15 and 18 weeks gestation), while controlling for relevant covariates. To test the hypothesis that PFC effects on thyroid hormone concentrations might differ in women with and without markers of autoimmune hypothyroidism, we also considered models with an interaction between PFC levels and elevated versus normal levels of thyroid peroxidase antibodies (TPOAb). In women with elevated TPOAb levels (10% of the study population), significant negative associations were detected between maternal fT4 and PFHxS, PFOS and SumPFASs. In the same women, positive relationships were detected between all measures of PFCs, except PFHxS, and maternal TSH. Regardless of TPOAb status, weaker positive trends were also found for PFNA, SumPFASs and TSH across the whole population. We hypothesize that women with elevated TPOAb, which is a marker of autoimmune destruction of the thyroid gland, may have reduced capacity to compensate for PFC-induced reduction of T4 compared to women with normally functioning thyroid glands (normal TPOAb). If true, PFCs may exacerbate the already lower maternal fT4 and higher TSH levels due to autoimmune hypothyroidism in up to 10% of pregnancies, or 45,000 pregnancies per year in Canada, with unknown effects on fetal development. These results await replication in larger, population-based studies.

**TP122 Sorption of Perfluorochemicals to Matrices Relevant to Sites Impacted by Aqueous Film-forming Foam** J. Sepulvado, Colorado School of Mines, Hydrology; C. Higgins, Colorado School of Mines, Environmental Science and Engineering. The presence of perfluorochemicals (PFCs) in groundwater as a result of the application of aqueous film-forming foams (AFFF) at fire training facilities has been documented. Concentrations of PFCs have been measured at these sites in the µg/L to mg/L range. Due to factors such as the recent push towards regulation of some PFCs in drinking water, recent concerns have arisen about the fate of PFCs in the subsurface. At fire training facilities, repeated applications of AFFF are used to extinguish fires ignited with hydrocarbon fuels. In some cases this has resulted in groundwater plumes containing PFC subclasses such as perfluorocarboxylic acids (PFCAs), perfluoroalkylsulfonates (PFAS), and fluorotelomer sulfonates (FtSs). These plumes also may contain co-contaminants such as hydrocarbon fuel components and chlorinated solvents, some of which may exist as nonaqueous phase liquids (NAPL). This study examined the sorption of PFCs to soil and aquifer material across the concentration range applicable to AFFF-impacted sites (µg/L – mg/L) and looked at the impact of co-contaminants, including NAPL, on PFC sorption. PFC sorption was variable and indicated that, similar to previous work on PFC sorption to sediment, subsurface PFC transport will depend on factors such as the sorbent, PFC subclass, solution chemistry, and PFC chain length.

**TP123 Gene Expression Profiles in Male Largemouth Bass Induced by Chronic Exposure to an Environmentally Relevant Level of 17α-ethinylestradiol** C.A. Richter, USGS – Biological Resources Division, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; D.M. Papoulias, US Geological Survey, Columbia Environmental Research Center; K.J. Kroll, Univ of Florida, Physiological Sciences; A. Mehinto, Univ of Florida, Dept of Physiological Sciences; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology; D.E. Tillitt, US Geological Survey, Columbia Environmental Research Center. Estrogenic endocrine disruptors are of increasing concern in aquatic systems. The occurrence of ovo-testis in male fish has been reported from diverse regions of the United States, and has been hypothesized to be related to estrogenic contaminants. To better understand the effects of long-term exposure to estrogens in fish, we studied gene expression profiles and physiological changes induced by chronic exposure to an environmentally relevant concentration (5 ng/L) of 17α-ethinylestradiol (EE2) in virgin adult largemouth bass in outdoor mesocosms. Vitellogenin production was increased and GSI was reduced in exposed males. However, reproductive success did not differ between exposed and unexposed treatment groups and no testes contained oocytes. Gene expression profiles in males were measured using a 15K largemouth bass microarray. The number of genes with altered expression in testes increased greatly between 3 and 15 months of exposure. Five potential biomarkers with strongly up-regulated expression in testes after both 3 months and 15 months exposure were identified. Gene expression in testes

and pituitary was highly responsive to EE2 after 15 months of exposure. Fewer changes in gene expression were observed in hypothalamus. In testis, after 3 months of exposure many genes related to regulation of the cell cycle were down-regulated. After 15 months of exposure, many genes related to aerobic production of ATP were down-regulated in testis and many genes related to protein synthesis were down-regulated in pituitary. The gene expression profiles observed in EE2-exposed males are consistent with delay of spawning readiness of the hypothalamus-pituitary-gonad axis in these fish.

**TP124 Stressor Induced Changes in *Lumbriculus variegatus* Transcripts and Metabolites** S.O. Agbo, Univ of Eastern Finland, Dept of Biology, Univ of Eastern Finland, Dept of Biology, Lab of Aquat Ecology & Ecotoxicology; J. Lemmetyinen, J. Akkanen, M.T. Leppanen, S. KeskiSaari, M. Keinanen, Univ of Eastern Finland, Dept of Biology; Z. Wang, H. Wang, Chinese Academy of Sciences, State Key Laboratory of Environmental Chemistry and Ecotoxicology; D.A. Price, Univ of Florida, The Whitney Laboratory for Marine Bioscience; J.V. Kukkonen, Univ of Eastern Finland, Dept of Biology. Exposures to contaminants can cause adverse effects that can be studied in organisms using classical life-history traits. The commonly used toxicity endpoints typically survival, mobility and reproductive success are some of the manifestations of the underlying molecular changes in organisms. Even though classical organism-level endpoints are realistic estimations of effects, they may not detect subtle changes and hence, are not suitable for use as early warning signs. Considering the widespread use of *L. variegatus* in ecotoxicology, extensive application of the OMICS technology is hampered by inadequate genome information. A combination of different methods may improve the understanding of contaminant mediated effects in relation to the underlying toxicity mechanisms. In this study, we report transcriptional changes in *L. variegatus* after waterborne exposures to B(a)P, cadmium, chlorpyrifos and pentachlorophenol (PCP). DNA damage was assessed by quantifying B(a)P Diol-Epoxyde (BPDE)-DNA adducts using capillary electrophoresis laser induced fluorescence immunoassay, after exposures to B(a)P. In general, genes with involvement in oxygen transport, mitochondrial function, reactive oxygen species and oxidative stress regulations, and heavy metal markers were mostly induced. Microarray data revealed that the relative change in transcript levels increased up to 16.6 folds for the selected genes within 6 h of PCP exposures, but decreased rapidly in a time dependent manner. The highest induction ratio was noted at 2 h B(a)P exposure but barely changed at 6 and 24 h prior to a rapid decline thereafter. Transcripts encoding alpha b, histone 3, thioredoxin peroxidase, glutathione s-transferase and metallothionein were the most consistent in expression, and may suggest that excess reactive oxygen species were generated due to B(a)P exposures. Majority of the significantly up-regulated transcripts were noted in treatments with B(a)P, whereas tests with chlorpyrifos showed the least differential expression. However, most of the changes in expression occurred early rather than later in exposure. The amounts of BPDE-DNA adducts suggest that DNA damage peaked within 6 h, and this corresponds with the observed transcriptional changes. Regardless of a few discrepancies in transcript abundance between microarray data and the corresponding quantitative PCR validation, expression patterns were comparable and consistent with the DNA adducts profile.

**TP125 Heart-specific Microarray Identification of AHR2-dependent and AHR2-independent Genes Involved in the Synergistic Developmental Toxicity of PAHs** L. VanTiem, J. Meyer, J. Meyer, Duke Univ, Nicholas School of the Environment; M. Kirby, Duke Univ, Dept of Pediatrics; K. Erwin, Duke Univ; H. Tsai, National Taiwan Univ; R. DiGiulio, Duke Univ, Nicholas School of the Environment. Numerous polycyclic aromatic hydrocarbons (PAHs) are teratogenic to fish. We have previously shown that binary co-exposures to a PAH that is an aryl hydrocarbon receptor (AHR) agonist and another that is a cytochrome P450 1 (CYP1) inhibitor result in synergistic developmental toxicity in fish embryos. The heart is a target organ for this toxicity, as pericardial effusion, impaired looping, elongated atrium and decreased cardiac output occur after these exposures. AHR2-morpholino (mo) knockdown prevents these deformities, providing evidence that AHR2 at least in part mediates PAH toxicity. However, the AHR2-dependent and AHR2-independent genes that control this synergistic toxicity remain largely unknown. In this study, microarrays were used to screen for the specific genes and pathways involved in the developmental toxicity caused by PAHs as well as genes dependent and independent of the AHR. We used zebrafish (*Danio rerio*) *cmic2::GFP* embryos that express GFP in the heart, allowing for easy heart extraction. Embryos were injected with either



an AHR2-mo or Control-mo (Co-mo). At 36 hours post fertilization (hpf), mo-injected embryos were dosed with DMSO, 100 µg/L benzo[a]pyrene (BaP, an AHR agonist), 500 µg/L fluoranthene (FL, a CYP1 inhibitor) or BaP + FL. At four time points (2, 6, 12 and 18 hours after dosing), hearts were extracted, and RNA extracted from pools of five hearts per treatment group was used for microarray analysis. Time points were chosen to capture early and secondary cell signaling events before onset of hatching and gross deformities. At 18 hpf, 189 genes were differentially expressed between Co-Mo BaP + FL and AHR2-mo BaP + FL treatment. Out of these genes, 183 were upregulated in the Co-Mo BaP + FL compared to the AHR2-mo BaP + FL treatment, and many of these genes are involved in cell adhesion, oxidation-reduction processes, calcium ion binding, zinc ion binding and positive regulation of apoptosis. Certain upregulated genes are also involved in various heart functions including cardioblast differentiation, angiogenesis and heart tube development. Data for the other time points are currently being analyzed. At 18 hpf, gene expression changes in response to BaP + FL appear to be mediated by AHR2. NIEHS-supported Duke Univ Superfund Research Program (P42ES10356) and ITEHP (T32ES07031).

**TP126 Biomarkers Screening and Proteomic Evaluation of Pesticides Toxicity on *Daphnia magna*** T. Lee, Chonbuk National Univ, Dept of Bioprocessing Engineering; S. Lee, Korea Research Institute of Chemical Technology, Toxicology Research Center; Y. Kim, Chungbuk National Univ, School of Life Science; J. Min, Chonbuk National Univ, Dept of Bioprocess Engineering. Proteomic analysis was performed to identify proteins involved in the stress responses of a water flea, *Daphnia magna*, to glyphosate and methidathion pesticides. Two lethal concentrations of pesticides including LC50 and LC75, which were determined by a 24h acute toxicity assay, were used for a 24h exposure to 21d daphnids prior to isolate the total protein. The proteomic profile of the testing organisms was analysed by the two-dimensional electrophoresis method with a 3-10 pH range. Three replicates were carried out for each testing concentrations and the average of the spot intensity was analysed in Progenesis program to explore the differentially expressed proteins (DEPs). The DEPs was considered as down- or up-regulations only if expression level of the protein linearly and significantly decreased C>LC50>LC75 or increased C.

**TP127 The Effects of the Ureic Based Herbicide Linuron on Reproductive Endpoints in the Fathead Minnow (*Pimephales promelas*)** A. Ornostay, Univ of New Brunswick Saint John, Dept of Biology and Canadian Rivers Institute; V.L. Marlatt, B.P. Lo, J. Elphick, C.J. Kennedy, Simon Fraser Univ, Dept of Biological Sciences; C.J. Martyniuk, Univ of New Brunswick Saint John, Dept of Biology and Canadian Rivers Institute. Linuron, a ureic-based herbicide, is widely used in crop production throughout North America and has been detected in aquatic environments at concentrations in the ng/L to µg/L range. Recent studies suggest that linuron has an anti-androgenic mode of action (MOA) in fish and mammals. However, additional research is required to better characterize the MOA and impact on the teleost reproductive axis. To examine the MOA of linuron, male and female fathead minnows were exposed to 100 ng/L 17-β estradiol (E2), 100 mg/L dihydrotestosterone (DHT), and 1, 10, and 100 mg/L linuron, and co-treatments of linuron and DHT (100 mg/L each) according to the Fish Screening Assay (Test No. 230, Organization for Economic Co-operation and Development). There were no effects of any treatment on gonadosomatic index, egg hatching or 7 day egg survival. Female fathead minnows showed a significant increase in nuptial tubercles with DHT and co-treatments of DHT and linuron. There were no changes in nuptial tubercles in males in any treatment. In female fathead minnows, DHT and linuron alone significantly reduced vitellogenin (Vtg) production while in males, E2 and co-treatments of DHT and linuron significantly increased Vtg production. Expression patterns were examined for a number of key genes involved in steroid production in the female ovary, including P450 side chain cleavage, aromatase, 3-β-hydroxysteroid dehydrogenase (3β-HSD), and 11-β-hydroxysteroid dehydrogenase (11β-HSD). There were no significant effects of linuron or model compounds on steroidogenic gene expression except for P450 side chain cleavage, which was decreased with co-treatment of DHT and linuron. Clustering of the expression patterns of the steroidogenic genes suggested that gene expression patterns were most similar between linuron and E2 compared to the model androgen DHT. This study suggests that linuron may have multiple MOAs that mimic either anti-estrogenic or estrogenic effects in a sex-specific manner in the fathead minnow teleost model.

**TP128 Using Genomics to Understand the Effects of Flame-retardant Chemicals on *Daphnia magna*** L.D. Scanlan, Univ of California, Berkeley, Molecular Toxicology, U.C. Berkeley, Molecular Toxicology, U.C. Berkeley, graduate student; E. Lachenauer, K. Dailey, X. Lin, C. Vulpe, U.C. Berkeley. Flame-retardant chemicals used in furniture, building materials and plastics are prevalent in and pose a threat to the environment. We are looking at the toxicity of banned "legacy" and new "replacement" flame-retardants on eco-indicator organism *Daphnia magna*. *D. magna* is an important fresh-water crustacean that is considered a keystone species in fresh-water ecology. We are using both traditional toxicity LC50 determination and genomic microarray and high-throughput sequencing technologies to determine the mode of toxicity of flame-retardants to *Daphnia magna*.

**TP129 Early Life Stage Salmonid Bio-monitoring in Polluted Environments Using Molecular and Physiological Bioassays** C.J. Martyniuk, Univ of New Brunswick, Dept of Biology/Canadian Rivers Institute, Univ of New Brunswick, Biology; R. Sherrard, Univ of New Brunswick; J. Elphick, V.L. Marlatt, Nautilus Environmental. Molecular bioindicators were developed to enhance an existing in situ early life stage (embryos to swim-up fry) cutthroat trout bioassay for use in diagnostic assessment of chemical stressors in the aquatic environment. This field study was conducted using cutthroat trout embryos to evaluate water quality associated with three sites on an urban stream in British Columbia, two sites of which were subjected to various anthropogenic inputs and a third considered a reference site. Endpoints included survival, deformities, size, vitellogenin, metallothionein (Mt) protein, and gene expression assays for well characterized transcripts that are responsive to metals (Mt A/B), endocrine disruptors (vitellogenin, estrogen receptors) and stress (cyp1A3, glutathione transferases). Vitellogenin (Vtg) levels in the head/tail protein extracts from the 3 field sites were not detectable, however low levels of *vtg* mRNA were detectable in the liver of fry. There was no detectable induction of *vtg* mRNA in the sites. Similarly, there were no significant differences in metallothionein protein levels in the gill between sites. These endpoints suggest that there were no significant estrogen/metal inputs into the streams. However, at the gene level, *cyp1A3*, *gsk* and zinc transporters were significantly increased in steady state abundance in these sites suggesting some stress and response to metals such as zinc. Furthermore, the impacts on estrogen and androgen receptor isoforms showed differential responses, indicating complex regulation of steroid isoforms in the environment. Cluster analysis of all genes examined in this study revealed the gene expression profiles in streams receiving municipal effluent were more similar to each other than to the reference site. We have optimized and demonstrated the utility of combined measures of reproductive success using both biochemical and molecular endpoints to assess the potential of a holistic approach in bio-monitoring using an early life stage salmonid test.

**TP130 Reproductive Effects of Sewage Effluents on Rainbow Darter (*Etheostoma caeruleum*) in Grand River, Ontario, Canada** P. Bahamonde, Univ of New Brunswick, Dept of Biology, Univ of New Brunswick, Biology Dept; G.R. Tetreault, Univ of Waterloo, Biology; M.R. Servos, Univ of Waterloo, Dept of Biology and Canadian Water Network, Univ of Waterloo, Dept of Biology; C.J. Martyniuk, Univ of New Brunswick, Dept of Biology/Canadian Rivers Institute, Univ of New Brunswick, Biology; M.E. McMaster, Environment Canada, National Water Research Institute; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology. Sewage effluents are a complex mixture between residential, institutional and industrial effluents. Chemicals with adverse effects found in sewage effluents can be Endocrine Disrupting Compounds (EDCs). Grand River (Ontario, Canada) is a model river to study the effects of sewage effluents on fish populations. Rainbow darter (*Etheostoma caeruleum*) is a small benthic fish found in North America, is very abundant and spatially distributed throughout the Grand River system. Furthermore, it is sensitive to sewage effluents, showing the presence of intersex condition in males. The primary objective of this research was to identify pathways involved in the formation of intersex condition and to explore the impact of sewage effluent on higher level biological endpoints. In October 2010 we collected female and male rainbow darter at 3 sites in Grand River. There were no changes in condition factor, no significant increase in hepatosomatic index (LSI), and significant decrease in gonadosomatic index (GSI), in fish in polluted environments compared with the reference site. Histology revealed that males had high incidence of intersex in polluted sites as characterized by the presence of follicles within the testis. To better

understand mechanisms underlying intersex in the rainbow darter, known genes involved in sexual differentiation and reproduction were cloned and are currently being evaluated with real-time PCR in males and females, such as vitellogenin, Sox9, foxl-2, and androgen receptors. In addition, a rainbow darter microarray has been developed using 454 pyrosequencing to further identify molecular pathways that are involved in intersex. Because the use of non-conventional species is getting more common and necessary for environmental monitoring, new tools are required to better understand the impact of anthropogenic impacts on river systems.

**TP131 Using Ingenuity Pathway Analysis® to Identify Early Developmental Networks Associated with ETBE and TAME Toxicity in Zebrafish (*Danio rerio*)** J.A. Bonventre, Rutgers, The State Univ of New Jersey, Joint Graduate Program in Toxicology; L.A. White, K.R. Cooper, Rutgers, The State Univ of New Jersey, Dept of Biochemistry and Microbiology. Gasoline additives ethyl tert butyl ether (ETBE) and tertiary amyl methyl ether (TAME) are used world wide to increase octane rating and burning efficiency of fuel. The consequence of developmental exposure to these hydrophilic chemicals is unknown for aquatic vertebrates. The effect of ETBE and TAME on zebrafish embryos was determined following OCED 212 guidelines. LC50s for ETBE and TAME were 14mM (95%CI=10 to 20) and 10mM (CI=8 to 12.5), respectively. A dose dependent increase in developmental lesions, including pericardial edema, abnormal vascular development, whole body edema, and craniofacial deformities, was observed with exposure to 0.625 to 10mM of either chemical. Similar lesions are associated with the dysregulation of matrix metalloproteinase (MMP) and Wnt protein families. We hypothesized that ETBE and TAME altered MMP and Wnt expression in the embryo. Exposure to 5mM ETBE or TAME decreased mRNA transcript levels of representative genes: *mmp2*, *mmp9*, and *wnt3a*, as well as structural component  $\beta$ -actin. ETBE reduced *mmp9* and *wnt3a* expression by 30 and 50%, but did not affect *mmp2*, while TAME decreased mRNA of both *mmps* by 70% and *wnt3a* by 60%. Both chemicals reduced  $\beta$ -actin by 50 and 70%, respectively. To better understand the impact of a reduction of these genes on development, a time course analysis of normal global gene expression patterns was conducted using the Zebrafish Affymetrix GeneChip®. Unexposed embryos were collected at 6-somites (15h), 21-somites (24h), and Prim-5 (30h), and processed for the microarray. Gene Spring® was used to determine genes that were differentially expressed from 15 to 24, and from 24 to 30h of development. Of the 1065 genes that were significantly altered, genes that had a greater than 2 fold change were entered into Ingenuity Pathway Analysis® software to determine the functional networks activated at each stage. From 15 to 24h MMPs and Wnts are involved in Cellular Assembly/Organization and Connective Tissue and Skeletal/Muscular system development networks. From 24 to 30h, these proteins are involved in Cellular Function/Maintenance and Molecular Transport networks. Using single gene endpoints for developmental toxicity studies provides a limited view of a chemical's toxicity. Understanding the networks that are involved in early development will help identify possible target pathways vulnerable to chemical insult, and may be used to direct future mechanistic studies.

**TP132 Connecting Molecular to Population-level Responses: Coupling Gene Expression to Dynamic Energy Budget Modeling** K.A. Gust, US Army, Engineer Research and Development Center, Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, ERDC-EL-EP-P; R. Nisbet, Univ of California, Santa Barbara; E. Muller, University of California, Santa Barbara; B. Ananthasubramaniam, Univ of California, Santa Barbara; M.S. Wilbanks, ERDC-EL-EP-P; A.J. Kennedy, US Army Engineer Research and Development Center; J.D. Laird, US Army Engineer Research & Development Center, SpecPro Incorporated, US Army Engineer Research & Development Center, Environmental Laboratory; B. Meeks, US Army Engineer Research and Development Center; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division. A significant challenge exists in bridging the rich data content resulting from contemporary genomics investigations to the prediction of population-level responses. As a means to tackle this challenge, we are coupling a dynamic energy budget (DEB) model (which has proven utility in predicting population dynamics) with gene expression data (specifically emphasizing responses in metabolic pathways related to energy metabolism and reproductive physiology). Our goal is to determine if bio-molecular

responses to stimuli can be used to predict population dynamics. Our dataset was generated utilizing *Daphnia magna* reproduction bioassays where sublethal lead (Pb) concentrations and food rations were varied to affect juvenile production. First, conventional methods for DEB modeling are being conducted in which the input parameter (ie. food) is modeled to predict allocation of assimilated material, energy to maintenance, growth and egg production. Genomic investigations are being conducted to determine the impact of Pb exposure and food ration at periods of time where the majority of surplus energy was expected to be dedicated to growth versus egg production and vice versa to determine the critical metabolic functions affected by these stimuli. Once the impacts on metabolic functions have been validated, net impacts of Pb exposure and food ration on metabolic output will be estimated and integrated into predictions of maintenance, growth and egg production in *Daphnia*. The predictive ability of the conventional approach to DEB modeling and the genomics-enabled DEB model will be compared to determine if inclusion of molecular responses improve model predictions. To the best of our knowledge, this is the first attempt at coupling gene expression data to predictions of population dynamics using a DEB approach.

**TP133 Global Metabolic Response in the Bile of Horned Frog (*Ceratotophrys cranwelli*) Tadpoles Exposed to Endosulfan** D.J. Marino, V. Sy, G.S. Natale, A.E. Ronco, Centro de Investigaciones del Medio Ambiente – UNLP, CONICET, Facultad de Ciencias Exactas, Universidad Nacional de la Plata; P. Carriquiriborde, Conicet, Centro de Investigaciones del Medio Ambiente, Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Química, Centro de Investigaciones del Medio Ambiente – UNLP, CONICET, Facultad de Ciencias Exactas, UNLP. The global metabolic response in the bile of horned frog (*Ceratotophrys cranwelli*) tadpoles exposed to endosulfan was assessed using UPLC-MS/MS. Tadpoles were exposed to 0, 5 and 10  $\mu$ g/L of endosulfan (a.i.) during 96 h. Treatments were performed by triplicate exposing seven organisms per replica. The gallbladders from five tadpoles per replica were pooled in a microtube with 1 ml of nanopure water, centrifuged and filtered through 0.22  $\mu$ m. Samples were analyzed using an UPLC system (ACQUITY UPLC®) with a C18 column (ACQUITY BEH) and couple to a tandem quadrupole mass spectrometer (Quattro Premier™ XE) equipped with an electrospray atmospheric pressure ionization interface (ZSpray™). Data were acquired using the MassLynx™ software and processed for peak detection, matching and alignment using the XCMS open access software. The highest number of ions was achieved in ESI negative mode (1122 peaks). The number of ions 10-fold up and down regulated in tadpoles exposed to 5  $\mu$ g/L, respect to the controls, was 64 and 59, respectively. In tadpoles exposed to 10  $\mu$ g/L the number of peaks was 90 and 57, respectively. Most of these ions belong to the same time clusters, suggesting they were fragments of the same metabolites. The strongest down regulation was observed for a peak at m/z 367, showing a clear concentration dependent response with 7,684 and 15,311 fold reduction at 5 and 10  $\mu$ g/L, respectively. The highest up regulation was observed for a peak at m/z 803, showing a concentration dependent response with a mean abundance of 0, 71 and 10,843 in 0, 5, and 10  $\mu$ g/L, respectively. A characteristic response at the global metabolic profile was observed in the bile of *C. cranwelli* exposed to endosulfan, which could be useful as fingerprint of the exposure to this organochlorine. In addition, further identification of major responding compounds could help to gain insight on the toxic mechanisms of the insecticide.

**TP134 Use of Network Inference to Unravel the Mechanisms of Action and Specificity of Aromatase Inhibitors** T. Habib, BTS; L. Escalon, US Army, Engineer Research & Development Center; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; D. Bencic, USEPA, Office of Research and Development, USEPA; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division; N. Garcia-Reyero, Jackson State Univ. The vertebrate hypothalamus-pituitary-gonadal (HPG) axis is controlled through various feedback mechanisms in order to maintain a dynamic homeostasis during changing environmental conditions, including exposure to chemical stressors. In this study, three aromatase inhibitors, fadrozole, prochloraz, and ketoconazole, were chosen as model chemicals to assess their effects on the hypothalamus-pituitary-gonadal (HPG) axis in the fathead minnows (*Pimephales promelas*). All three chemicals have

been shown to inhibit aromatase activity and decrease estradiol (E2) levels. Fadrozole, a drug used to treat breast cancer, is a potent aromatase inhibitor. Prochloraz, an imidazole fungicide, affects multiple pathways, such as inhibition of aromatase and other CYPs, as well as antagonism of the androgen receptor. Ketoconazole, an anti fungal agent, affects multiple pathways as well, including inhibition of aromatase and other CYPs. We exposed female fathead minnows to two different concentrations of fadrozole (3, 30 ug/L), prochloraz (30, 300 ug/L), and ketoconazole (32, 310 ug/L) during 8 days. We then removed the chemical from the water and sampled for 8 more days. We analyzed plasma hormone levels, as well as gene expression changes in the ovaries using a 15k custom microarray. We used network analysis to try to elucidate the mechanisms of action of the chemicals and their degree of specificity as aromatase inhibitors. These chemicals significantly increased aromatase expression and reduced E2 production. Comparison of the three aromatase inhibitors based on the network inference showed that fadrozole acted as the strongest aromatase inhibitor. Therefore, microarray analysis supported the fact suggested by hormone levels that fadrozole is a more potent aromatase inhibitor than prochloraz and ketoconazole. The inferred network was also able to discern between fadrozole, a more specific aromatase inhibitor, prochloraz, and ketoconazole, the last two being more general P450 inhibitors. The results also unraveled new connections that have been verified in the literature.

**TP135 Toxicity Mechanism-based Classification of Environmental Contaminants Based on Transcriptional Level Cellular Stress Response Profiling** A. Gu, Northeastern Univ, Dept of Civil & Env Engineering, Biotechnology Initiative Program, Dept of Civil & Env Engineering, Biotechnology Initiative Program; C. Gao, Northeastern Univ, Civil and Environmental Engineering; N. Gou, A. Onnis-Hayden, Northeastern Univ. The needs to evaluate the toxicity of an large and ever-increasing number of chemicals demand for development of toxicity mechanism and pathways-based cost-effective testing scheme, then in time we hope to eventually build predictive models and tools to provide toxicological information that transcends the limits of data generation. The advance in “-omics” technologies present promises for achieving this goal. In this study, we utilized a whole-cell array of *Escherichia coli* K12 strains carrying gene promoters with transcriptional *gfp* fusions, covering most known stress response-related genes, to develop a methodology for quantitative transcriptional level ecotoxicity assessment and classification of toxicants. We examined 11 toxicants including model chemicals such as Mitomycin C (MMC) and H<sub>2</sub>O<sub>2</sub>, and various contaminants such as 4-Nonylphenol (4-NNP), nano-silver (nAg) and single-walled nanotube (SWNT) at various dose concentrations. The real-time gene expression levels over the exposure of 2 hours was recorded, and transformed into a newly developed Transcriptional Effect Level Index (TELI) to quantify the molecular toxicological impacts. Classification and clustering of these compounds at different doses were conducted and compared based on both quantitative stress response TELI values patterns across different stress pathways as well as, based on 3-D temporal pattern and magnitude of altered gene expression levels. The results indicated that there were consistencies as well as discrepancy of the clusters formed among the materials, depending on the different clustering logic and methods used (Hierarchical Clustering (HCL) and Self-Organizing Maps (SOM)). In general, the same chemical of various dose concentrations or, chemicals sharing similar toxic mechanisms seemed to cluster together. Clustering using simplified stress response category patterns seemed to cluster more “logically” with HCL approach, whereas, clustering using more complex temporal altered expression profiles yielded more “reasonable” clustering with SOM approach. In conclusion, we have demonstrated that transcriptional level effects can be quantified and it exhibited dose-response patterns. And, classification of toxicants based on their stress response profiling, meaning their potential toxicity mechanisms, are possible, although the clustering results vary depending on the data and approach used.

**TP136 Validation of a LC/MS/MS Method for Measuring BPA, BP-3, Triclosan, Parabens, and other Environmental Phenols in Human Urine** Q.W. Gavin, State of California, Dept of Public Health, Environmental Health Laboratory Branch; R. Ramage, P. Behniwal, J. She, State of California, Dept of Public Health. Bisphenol A (BPA) is used in the manufacture of food packing, drinking containers, dental sealants, and medical devices. 2-hydroxy-4-methoxybenzophenone (BP-3) is used as sunscreen agents for skin protection, and as UV filters in cosmetic products and plastics to improve stability. Parabens and triclosan are widely used as preservatives

and antimicrobial agents, respectively, in personal care products, pharmaceuticals, and food processing. Chlorophenols have been used in the wood preservation industry, as intermediates in the production of pesticides, and as disinfectants or fungicides for industrial and indoor home use. Because of their widespread use and potential risk to human health, environmental phenols were selected as high priority chemicals to be monitored by the California Environmental Contaminant Biomonitoring Program (Biomonitoring California). Therefore, we need to develop and validate a reliable, specific, and accurate method. A sensitive HPLC-isotope dilution-MS/MS method was developed and validated using QTRAP 5500 to simultaneously measure the concentrations of BPA, BP-3, 4-tert-octylphenol (tOP), ortho-phenylphenol (OPP), triclosan, parabens (methyl-, ethyl-, propyl-, and butyl- parabens), and four environmental chlorophenols; 2,4-dichlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol in human urine. Prior to LC/MS/MS, analytes are preconcentrated by solid-phase extraction (SPE) on Varian C18 cartridges. This simple SPE procedure provides extraction recoveries above 85%. The method was validated using Quality Control samples prepared from pooled human urine at three spiking levels. Intra and inter-batch precision and accuracy assessments show good reproducibility (inter-day coefficient of variations ranging from 3.82% to 14.2%) and accuracy (spiked recoveries ranging from 90% to 103% at the three spiking levels). The detection limits for most of the analytes are below 1 ng/ml in 1 ml of urine. This method will be used and applied for analyzing samples in Biomonitoring California.

**TP137 An Evaluation of Adducts of Cysteinylglycine as Potential Biomarkers of Exposure to Formaldehyde and Acetaldehyde from Cigarette Smoke** E. Minet, British American Tobacco, R&D Center; G. Scherer, Analytisch-Biologisches Forschungslabor GmbH. Introduction: The WHO Study Group on Tobacco Product Regulation (TobReg) recommended a list of toxicants in mainstream smoke of cigarettes for mandated lowering. This list comprises 9 tobacco smoke constituents, including acetaldehyde and formaldehyde. A reduction in exposure to those toxicants, through the use of new filters and tobacco technologies, can be assessed with suitable biomarkers of internal dose measured in biofluids such as urine. As yet, no biomarkers for the smoking-related exposure to these two aldehydes are available. Previous investigations suggested that acetaldehyde forms a stable adduct (2-methyl-thiazolidine-4-carbonyl-glycine (MTCG)) with the cysteinylglycine (CysGly) dipeptide, which is a degradation product of glutathione (GSH). By analogy to acetaldehyde, we hypothesized that formaldehyde can also form a stable adduct with CysGly (thiazolidine-4-carbonylglycine (TCG)). However, MTCG and TCG have not been identified in urine of any species, including humans. Objective: The objective of this project was to develop an analytical method for the determination of MTCG, a biomarker for acetaldehyde, and TCG, a biomarker for formaldehyde, in human urine. Method: An LC-MS/MS method, comprising a derivatization step with propylchloroformate followed by organic extraction, was developed and applied to a urine sample series obtained from non-smokers (n=50) and smokers of 1 mg (n=46) and 6 mg (n=49) ISO tar yield cigarettes. Nicotine and 5 of its metabolites were also quantified to establish correlations between TCG, MTCG, and tobacco consumption. Results: Our results show that, for the first time, MTCG (0.72±0.45 ng/ml) and TCG (13.76±9.58 ng/ml) were detectable in human urine. No significant difference was recorded between smokers and non-smokers, and no correlation could be established with total nicotine measured in the urine of smokers. The high TCG and MTCG background observed in non-smoker urine indicates the interference of possible confounding factors at low level exposure. However, preliminary results indicate that MTCG could be a suitable biomarker for acetaldehyde as a by-product of ethanol consumption, and should be explored further.

**TP138 Elimination of Pyrethroid Pesticides and Pyrethroid Metabolites from Brain of Dosed Rats** J. Starr, D. Ross, C. Cunningham, M.F. Hughes, USEPA. Pyrethroids are an important class of insecticides. In mammals, pyrethroids are neurotoxic and generally act by prolonging the opening of voltage sensitive sodium channels. Established kinetic models of pyrethroids have been used to assess toxicity and to interpret biomonitoring data. Most studies have relied on parent depletion to establish kinetic rates, and have not directly measured product (metabolite) formation. The purpose of this research was to develop profiles for common pyrethroids and their oxidative metabolites in brain tissue. Rats were dosed orally with a pyrethroid mixture containing cypermethrin, deltamethrin, esfenvalerate,



cis/trans-permethrin, and cyfluthrin. The animals were sacrificed and brain samples taken at 2.5, 3.5, 5.5, 9.5 and 25.5 hours post dosing. Analytes included the parent pyrethroids and the following metabolites: cis/trans-dichlorovinyl-dimethylcyclopropane-carboxylic acid (DCCA), 3-phenoxybenzoic acid (3PBA), 4-fluoro-3-phenoxybenzoic acid (4F3PBA), and cis-dibromovinyl-dimethylcyclopropane-carboxylic acid (DBCA). Analytes were extracted with hexane:acetone (8:2, V:V), cleaned using a silica solid phase, then analyzed by liquid chromatography, tandem mass spectrometry. Maximum concentrations of the pyrethroids were reached at or before the 3.5 hour time point. By 25.5 hours, concentrations of almost all parent pyrethroids were below the detection limit. DBCA was not present in quantifiable amounts in any sample. The other metabolites were more persistent than their parent pyrethroids. Since the metabolites were eliminated from the brain more slowly than the parent pyrethroids, their concentrations relative to their respective precursors increased. The concentration of DCCA was low compared to 3PBA and 4F3PBA, however, the half-life of DCCA was longer than both 3PBA and 4F3PBA. Pyrethroid metabolites may be formed by enzymatic activity in the brain, liver, or serum. Therefore, their presence in the brain may be largely due to transport of the metabolites across the blood/brain barrier. Despite this, the observed difference in elimination rates between parents and metabolites shows the brain to be an important physiological compartment. These results can be used to more fully parameterize toxicokinetic models describing the clearance of pyrethroids. These models will be useful in risk assessment as well as exposure reconstruction studies where estimates of exposure are often based on urinary biomarker measurements.

**TP139 Fast and Simultaneous Determination of Urinary 8-hydroxy-2'-deoxyguanosine and Monohydroxylated Polycyclic Aromatic Hydrocarbons** R. Fan, Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, College of Life Science, South China Normal Univ, California Dept of Public Health, Environmental Health Laboratory Branch; D. Wang, California Dept of Public Health, Environmental Health Laboratory Branch; R. Ramage, J. She, California Dept of Public Health, Environmental Health Laboratory Branch. 8-hydroxy-2'-deoxyguanosine (8-OHdG), as a biomarker of oxidative DNA damage, has been extensively studied to assess human exposure to those carcinogenic compounds. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. The published studies have associated levels of human urinary hydroxylated PAHs with those of 8-OHdG. However, the measurement of hydroxylated PAHs and 8-OHdG in urine was often carried out by using two different analytical methods, which consumed longer time and high cost. In this study, a novel method was developed to quantify 10 urinary hydroxylated OH-PAHs and 8-OHdG within 11min by liquid chromatography/tandem mass spectrometer simultaneously. Deuterated and <sup>13</sup>C- labeled analogs were used as internal standards. The urine samples went through solid phase extraction and concentration, and were analyzed by an optimized LC/MS/MS method operated in the negative ESI and MRM mode. The method detection limits (MDLs) in pooled urine ranged from 0.023 µg/L to 0.625 µg/L. The calibration curves of all target analytes showed good linearity within the concentration range of 0.31-5.00 µg/L for 8-OHdG and 0.047-15.00 µg/L for different OH-PAHs. The method showed satisfied accuracy and precision by analyzing varied levels spiked pooled urine. The recoveries were in the range of 84%-115% with variation coefficient of less than 20%, except for 2-OHF and 1-+9-OHPhe.

**TP140 Modulation of Thyroid Hormone Concentrations in Rats Co-administered with Perchlorate and Iodide-deficient Diet** T. Kunisue, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany, Research Scientist, New York State Dept of Health, Research Scientist; K. Kannan, Wadsworth Center, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany; J.W. Fisher, Dept of Environmental Health Science, College of Public Health, Univ of Georgia. Perchlorate can perturb thyroid hormone (TH) homeostasis by competitive inhibition of iodide uptake by the thyroid gland. Until recently, the effects of perchlorate on TH homeostasis were examined by measuring serum concentrations of THs by immunoassay (IA) methods. IA methods are sensitive, but for TH analysis, they are compromised by the lack of adequate specificity. In this study, we determined the concentrations of six THs: L-thyroxine (T4), 3,3',5-triiodo-L-thyronine (T3), 3,3',5'-triiodo-L-thyronine (rT3), 3,5-diiodo-L-thyronine (3,5-T2),

3,3'-diiodo-L-thyronine (3,3'-T2), and 3-iodo-L-thyronine (3-T1) in the serum of rats administered perchlorate by isotope (<sup>13</sup>C6-T4)-dilution liquid chromatography (LC)-tandem mass spectrometry (MS/MS). The method recoveries for THs spiked into a serum matrix were between 97.0% and 115%, with a coefficient of variation (CV) of 2.1% to 9.4%. Rats were placed on an iodide-deficient or iodide-sufficient diet for 2.5 months, and for the last 2 weeks of that period, they were provided drinking water either without or with perchlorate (10 mg/kg bw/day). No significant differences in serum concentrations of T3 and T4 were observed between rats given iodide-deficient and iodide-sufficient diets for 2 or 2.5 months. After 24 h of perchlorate exposure, significantly lower concentrations of T3 and T4 were found in the serum of rats administered the iodide-deficient diet but not in rats administered the iodide-sufficient diet. However, following 2 weeks of perchlorate exposure, TH levels in rats fed with the iodide-sufficient diet were also significantly lower than those in control rats. Our results suggest that perchlorate affects TH homeostasis and that such effects are more pronounced under iodide-deficient nutrition.

**TP141 Occurrence of Benzotriazole UV Stabilizers and Synthetic Musks in Human Adipose Tissues Collected from Japan, South Korea, China, Spain, and the USA** H. Yanagimoto, Kumamoto Univ, JAPAN, Kumamoto University; H. Nakata, Kumamoto Univ, Graduate School of Science and Technology, Kumamoto Univ, Dept of Environmental Science; R. Shinohara, Kumamoto Univ, Graduate School of Science and Technology; T. Isobe, Ehime Univ, Senior Research Fellow Center, Ehime Univ, Senior Research Fellow; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies; N. Nose, H. Komori, N. Arita, N. Ueda, Ehime Univ; M. Watanabe, National Institute for Environmental Studies; B. Jemenez, Spanish Council for Scientific Research; J. Yang, Catholic Univ of Daegu, Dept of pharmacology and toxicology; T. Kunisue, New York State Dept of Health, and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany, Research Scientist, New York State Dept of Health, Research Scientist; K. Kannan, New York State Dept of Health, Wadsworth Center. The benzotriazole UV stabilizers (BUVSs) and synthetic musks have been used in a wide variety of plastic materials and personal care products, respectively. In recent years, ubiquitous contamination by BUVSs and musks has been reported in the aquatic environment. These compounds accumulate in higher trophic species, such as marine mammals and coastal birds, which indicate their bioaccumulation potential through the marine food-chain. Despite this, very few studies have examined the occurrence and concentrations of BUVSs and synthetic musks in human adipose tissues. In this study, we analyzed four BUVSs, such as UV-320, UV-326, UV-327 and UV-328 and five synthetic musks, such as HHCB, AHTN, Musk-xylene, Musk-ketone and OTNE in human adipose tissues collected from Japan, South Korea, China, Spain, and the USA. UV-327, UV-328, HHCB, and AHTN were detected in most samples analyzed. High concentrations of BUVSs were found in adipose tissues collected from Japan and South Korea, at the levels of several tens of ng/g on a lipid wt. basis. HHCB showed higher concentration in samples from Spain and the USA. Significant correlation between UV-327 and UV-328 concentrations was found in human tissues, implying that these compounds might have originated from similar sources. The profiles of geographic distribution of BUVSs and synthetic musk concentrations in human tissues were similar to that of mussels collected from the Asia-Pacific region. No gender- and age-related differences were observed in the concentrations of BUVSs and musks in human tissues. These results were different from that for persistent organic pollutants (POPs), which indicate that biodegradation and metabolism of these compounds. To our knowledge, this is the first study to report the occurrence bioaccumulation and widespread contamination of BUVSs in human adipose tissues.

**TP142 Optimization and Validation of a New Analytical Method for the Monitoring of Metabolites of Organophosphate Flame Retardants and Plasticizers in Urine** N. Van den Eede, Univ of Antwerp, Pharmaceutical Sciences; A.C. Dirtu, Univ of Antwerp, Pharmaceutical Sciences, "Al. I. Cuza" Univ of Iasi, Chemistry; H. Neels, Univ of Antwerp, Pharmaceutical Sciences; A. Covaci, Univ of Antwerp. Since 2004, the demand for alternative flame retardants has increased as a result of the ban on the use of Penta- and Octa-BDE mixtures by the European Union. Organophosphate esters (OPEs) are one type of such alternatives and have become widely used since then. Now they are largely present in the indoor environment, mainly in air (ng/m<sup>3</sup>) and dust (µg/g). Human exposure to these chemicals may

be therefore high, both for adults, who are mainly exposed via inhalation, and for toddlers who are exposed via inhalation and dust ingestion. OPEs are hydrolyzed in the liver to form dialkyl phosphates or diaryl phosphates (DAPs), depending on the structure of the parent compound. These DAPs can be traced in human urine and can serve to monitor human exposure to OPEs. We aimed at developing an analytical method for the quantification of six DAPs in human urine, namely bis(1-chloro-2-propyl) phosphate (BCPP), bis(2-butoxyethyl) phosphate (BBEP), bis(2-chloroethyl) phosphate (BCEP), bis(1,3-dichloropropyl) phosphate (BDCPP), diphenyl phosphate (DPP) and di-(*iso/n*-)butyl phosphate (DBP). Sample preparation was based on solid phase extraction using a weak anion exchange sorbent (Varian NH<sub>2</sub>, 1 ml/50 mg) and elution with 5% ammonium hydroxide in methanol. Chromatographic separation was achieved on an Agilent 1200 series LC using a Kinetex HILIC 150 mm x 2.1 mm x 2.6 µm column, coupled to an Agilent 6410 triple quadrupole mass spectrometer employed in electrospray negative ionization mode. DAPs were quantified on four labeled internal standards, namely BCEP-d8, DPP-d10, BDCPP-d10, BBEP-d4, using multiple reaction monitoring. The method was optimized using spiked urine and further validated by matrix spiking at 3 concentrations. Matrix effects were assessed for both DAPs and their corresponding internal standards. The method is currently employed in various biomonitoring studies, with the aim to characterize the human exposure to OPEs.

**TP143 Urinary Bisphenol-A and Triclosan Levels in a Belgian Obese Population During a 12-month Follow-up Study** T. Geens, Univ of Antwerp, Pharmaceutical Sciences; A. Dirtu, Univ of Antwerp; E. Dirinck, Univeristy Hospital of Antwerp; H. Neels, Univ of Antwerp; L. Van Gaal, P. Jorens, Univeristy Hospital of Antwerp; A. Covaci, Univ of Antwerp. Bisphenol-A (BPA) and triclosan (TCS) are two phenolic chemicals with endocrine disrupting properties. Distribution of these compounds in the environment is widespread (water, soil, fish, dust) and almost all humans in occidental countries are exposed. For BPA, food is the most important exposure pathway, while for TCS, the use of personal care products is the most dominant source. Due to their fast biotransformation and urinary excretion of their conjugated form, urine is the most appropriate matrix for biomonitoring of these compounds. In this study, BPA and TCS were determined in a group of obese patients visiting the Obesity and Diabetes Clinic of the Univ Hospital of Antwerp, Belgium. At the start of this weight loss program, about one-third of the patients underwent bariatric surgery followed by diet recommendations and physical exercise, while the other two-third was treated with only diet recommendations and physical exercise. Urine spot samples were collected at the start of the program (N=102) and after approximately 3 months (N=57), 6 months (N=33) and 12 months (N=10) during follow-up visits. The analytical procedure included a deconjugation step, after which BPA and TCS were extracted from 3 mL urine by solid phase extraction with Oasis HLB (60 mg, 3 mL) followed by derivatization with pentafluorobenzoylchloride and detection with gas chromatography coupled to negative ionization mass spectrometry. The method limit of quantification was 0.1 µg/l for both contaminants analyzed. Both BPA and TCS were quantified in more than 99% of the samples. Overall, BPA ranged between 0.1 and 13.2 µg/L with a median concentration of 1.42 µg/L, while TCS showed a wider concentration range of 0.1 – 1048 µg/L with a median of 1.49 µg/L (75<sup>th</sup> percentile: 5.4 µg/L and 90<sup>th</sup> percentile: 132.6 µg/L). Levels of BPA were more constant at the different time points, indicating a more uniform exposure. The presence of outliers for TCS, on the other hand, is more indicative for spikes in exposure. Urinary concentrations were creatinine-adjusted and correlated with a set of clinical parameters. Since the changing of the diet might have an influence on the exposure to BPA, time-trends were examined in relation with the type of diet, also taking into account factors such as weight loss, BMI, etc.

**TP144 Air-Water Gas Exchange of Legacy and Currently Used Pesticides in the Arctic** L.M. Jantunen, Environment Canada; F. Wong, Univ of Toronto, Chemistry; A. Gawor, Univeristy of Toronto; T.F. Bidleman, Environment Canada; G. Stern, Freshwater Institute, Dept of Fisheries and Oceans; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; H. Kylin, Swedish Univ of Agricultural Sciences, Aquatic Sciences and Assessment. The arctic has been contaminated by legacy organochlorine pesticides (OCPs) and currently used pesticides (CUPs) through atmospheric transport and oceanic currents. Although CUPs are less persistent than OCPs, they are still detected in the arctic air and water. Endosulfan is the most reported CUP in the arctic environment

but little is known about other CUPs especially in the ocean water, although several have been found in arctic lakes, snow and ice. The spatial trends and air-water exchange of these pesticides were studied by collecting parallel water and air samples from shipboard expeditions in sub-arctic and arctic regions between 1993-2010. Over the many years, similar air and water sampling and analytical methods were employed, ensuring data comparability. Compounds found in this study include the legacy OCPs: hexachlorocyclohexanes (HCHs), chlordanes, dieldrin and toxaphene and the CUPs: dacthal, endosulfans, chlorothalonil, chlorpyrifos and trifluralin. Based on two samplings in 1999 and 2007/08, concentrations of the legacy compounds HCHs and chlordane are declining in Canadian Archipelago water, whereas some CUPs, notably endosulfan, are increasing. Within the Canadian archipelago there is also an east-west spatial gradient, most notably for the HCHs, dieldrin, dacthal and endosulfan. Gas exchange varies by compound, year and location, but generally in the Canadian Archipelago, legacy OCPs are undergoing net outgassing where CUPs are undergoing net deposition. Understanding the processes and current state of air-surface exchange is important in order to assess human and environmental exposure, evaluate the effectiveness of International Protocols and in turn provide insights for new and emerging chemicals.

**TP145 Contribution of Air-to-Surface Partitioning to Deposition Potential for Airborne Cyclic Methylsiloxanes: A Modeling Assessment** S. Xu, Dow Corning Corporation, Health and Environmental Sciences. Partition of airborne contaminants to surface media such as soil, biota, aquatic suspended particulates, and sediment have been identified as significant contributors to deposition potential for known persistent organic pollutants (POPs) such as PCBs and pesticides. In this study, the effects of partitioning of airborne cyclic volatile methylsiloxanes (cVMS) to soil, plant biomass, suspended particles and aquatic sediments in the remote region on the deposition potential of cVMS were estimated by equilibrium modeling. For each process, partitioning equilibrium between surface medium and air was assumed and the partitioning coefficients were estimated based on available data and approaches reported in literature. It was found that cVMS have surface media/air partition coefficients 3 to 6 orders of magnitude lower than those of the most volatile POP reference materials such as hexachlorobenzene (HCB) and PCB 28 congener. This is especially true for D4 and D5. Consequently, gas absorption via soil/air, plant/air, suspended particulates/air, and air/water/sediment partitioning contributes minimally (< 1% of total airborne D4 or D5) to the deposition potential of D4 and D5 in the remote region. For D6, partition from air to plant biomass, suspended particulates and sediment contribute minimally to the deposition of airborne D6 to surface media, while soil/air partitioning may account for a small fraction (e.g., 3%) of airborne D6 transferred from air to soil under the low temperatures (e.g., -20 oC) in the remote region.

**TP146 Are the Electronic Wastes and Wastes Generated from Ship Breaking Sites Increasing the Atmospheric Load of Polychlorinated Biphenyls in India?** P. Chakraborty, K. Sajwan, Savannah State Univ, Dept of Natural Science; G. Zhang, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences; K. Jones, Institute of Environmental and Natural Sciences, Lancaster Univ, Lancaster, Dept of Environmental Sciences. Asia has become hotspots of polychlorinated biphenyls (PCBs) though Asia was not identified as an important source in global emission inventories of PCBs. There are growing concerns that most of the electronic waste (e-waste) generated in developed countries is ending up in developing countries that are economically challenged and lack the infrastructure. India is of prime concern because tropical climate of India would facilitate the rapid entry of PCBs to the atmosphere from some sources such as incineration of PCB containing materials, vaporization from landfill, air-water/soil exchange, vaporization from contaminated surfaces, sludge dewatering beds, and electronic devices containing PCBs. Incomplete combustion of e-waste in open air and dumping of processed materials are the major sources of various toxic chemicals including PCBs. Co-disposal of e-waste with domestic waste in open dumps is generally practiced in India. Another important source for PCBs in India may include dumping of PCB-containing wastes with release via volatilization and uncontrolled burning, and the storage and breakup of old ships. India is world's largest ship breaking nation in terms of volume. Most of the ships dismantled today were built in the 1970s. That is to say prior to the banning of many hazardous materials. Presently atmospheric levels of PCBs in India seem to have largely increased since early 1990s. A predominant

gradient in the maximum concentration of  $\Sigma 25$ PCB has been observed between urban-rural-wetland/background with an exceptionally higher level in the wetland region of Sunderban (544 pg.m<sup>-3</sup>). PCBs in the Indian atmosphere showed a dominance of congeners with 5-7 chlorine atoms (heavy PCBs), and dioxin-like PCBs were significant in the descending order of Mumbai, New Delhi, Agra, Kolkata, and Chennai. The metro-cities are of prime interest in this case because of the electronic waste generated with the rapid growth of improper electronic waste recycling units. Elevated levels observed in the wetland regions of Sunderban highlights regional atmospheric transport of PCBs from major cities to remote/pristine sites in India.

**TP147 Estimating Hourly Passive Sampler Mass Transfer Rates and Concentrations in an Urban Landscape Using a Numerical Weather Prediction Model** N.T. Petrich, Univ of Iowa, Environmental Engineering. Nicholas T. Petrich, Scott N. Spak, Dingfei Hu, Zach L. Rodenburg Gregory R. Carmichael, Keri C. Hornbuckle Polyurethane foam (PUF) passive air samplers represent an inexpensive and practical way to sample semivolatile organics compounds and their spatial gradients over urban areas. However, canonical methods for estimating concentrations from passive sampling assume constant empirical mass transfer rates and temperature, which add unquantified uncertainties in temporal representativeness to concentrations estimated from raw sampled mass. These methodological choices currently limit the ability to calculate and constrain uncertainty in estimated concentrations, and to directly compare passive sampler concentrations with time-resolved concentrations from active sampling methods and chemical transport models. Here we present an alternative method for estimating hourly flow rates, mass transfer coefficients, and concentrations—including average concentration at a reference temperature—from first principles using hourly meteorology (temperature, wind direction, and wind speed) simulated by the Weather Research and Forecasting (WRF) model. This method is applied to gas and aerosol polychlorinated biphenyl (PCB) congeners at an urban passive sampling network in Chicago, Illinois during 2008. We evaluate the importance of sampler height and orientation, temperature, sub-gridscale turbulent mixing, internal fluid dynamics within the PUF housing, and PUF air concentration gradients. Effects of model spatial resolution are considered using nested WRF simulations on 12 km, 4 km, and 1.33 km grids over the city. Spatial variability in mass transfer coefficients and concentrations are compared with results from canonical empirical methods, and temporal variability evaluated through comparison with coterminous concentrations from high volume air samplers.

**TP148 Halogenated Polycyclic Aromatic Hydrocarbons in Urban Air from Albany, USA** Y. Horii, Center for Environmental Science in Saitama; T. Ohura, Meijo Univ; B.R. Johnson, Univ of Cartagena; K. Kannan, Wadsworth Center. Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants. Chlorinated PAHs (ClPAHs) and brominated PAHs (BrPAHs) are structurally similar to other halogenated hydrocarbons such as dioxins. Cl-/BrPAHs with three to five aromatic rings have been reported to occur in various environmental matrices. Recent synthesis and purification of individual halogenated PAHs in our laboratory made the congener-specific analysis of Cl-/BrPAHs possible. Previous studies have focused on occurrence, profiles, and atmospheric behavior of Cl-/BrPAHs in urban air from Japan. In this study, we determined individual concentrations of 26 ClPAHs and 15 BrPAHs in gaseous and particulate phases of urban air collected in Albany, NY, USA. We also investigated seasonal changes of Cl-/BrPAHs and the correlation between Cl-/BrPAH and the corresponding parent PAH concentrations to understand the atmospheric behavior of these compounds. This is the first report to analyze Cl-/BrPAHs in American air samples. Monitoring was conducted every month from June, 2007 to May, 2008 at the Empire State Plaza located in Albany, NY. Two days of air samples were collected using high-volume air sampler attached with quartz fiber filter (QFF) and polyurethane foam (PUF) and XAD-2 adsorbent sandwich (ORBO 2500Puf sandwich, Supelco). Concentrations of ClPAHs, BrPAHs, and PAHs in the QFF and PUF sandwich samples were determined using a GC/HRMS. Concentrations of sum of ClPAHs varied from 0.29 pg/m<sup>3</sup> to 5.6 pg/m<sup>3</sup> in gaseous phase and from 0.11 pg/m<sup>3</sup> to 4.8 pg/m<sup>3</sup> in particulate phase. The mean concentrations of target chemicals in total of gaseous and particulate phases decreased in the following orders: PAHs (3800 pg/m<sup>3</sup>) > ClPAHs (3.2 pg/m<sup>3</sup>) > BrPAHs (0.089 pg/m<sup>3</sup>). Significant correlation between the concentrations of Cl-/BrPAHs and the corresponding PAHs was observed in total of gaseous and particulate phases. The concentrations of low molecular weight Cl-/BrPAHs (e.g., 9-ClPhe) in gaseous phase were

3-5 times higher than those in particulate phase, while the concentrations of high molecular weight Cl-/BrPAHs (e.g 6-ClBaP) were higher in particulate phase. Although seasonal trends of ClPAHs in urban air from Japan which showed higher concentrations of ClPAHs in colder seasons, were found in previous studies, the seasonal trend was not clearly shown in our results. The concentration levels of Cl-/BrPAHs in the urban air from Albany were one order of magnitude lower than those from Japan.

**TP149 Pesticides in Air in the Mississippi River Valley: A Comparison Between 1995 and 2007** W.T. Foreman, US Geological Survey, National Water Quality Laboratory; P.D. Capel, M.S. Majewski, S.J. Kalkhoff, R.H. Coupe, R.C. ReVello, US Geological Survey. Weekly-composite air samples were collected using a glass-fiber filter/polyurethane foam plug high-volume air sampler during the 1995 and 2007 April through September growing seasons at two agricultural locations in Iowa and Mississippi. Samples were analyzed by GC/MS using selected-ion monitoring for a variety of pesticides and degradates, 31 of which overlapped the two sampling periods. At least 26 compounds were detected during the growing season from each site in both years. The number of pesticides detected in more than 50% of the samples doubled for 2007 compared to 1995. Pesticide use patterns (amounts, crop types, application procedures), coupled with seasonal weather and atmospheric processes influence detection frequencies and aerial concentrations. For example, trifluralin was detected nearly 100% of the time at both sites during both years; likely because it is used on a variety of different crops and is incorporated into the soil prolonging its period of volatilization following initial application. Atrazine (and its primary degradate chloroisopropylamino-s-triazine; CIAT) and metolachlor, which are extensively applied at both locations, were detected at least 50% of the time at all sites during both years. The insecticide chlorpyrifos increased in detection frequency from about 38% to 95% at both sites in 2007 compared to 1995; its concentration was greater in 2007 than 1995 in Iowa, but was comparable between the two years in Mississippi. Similarly, the pyrethroid insecticide cis-permethrin was detected more frequently and had greater concentrations at both sites in 2007 compared to 1995 that likely is a result of replacement of some use-restricted or eliminated organophosphorus or carbamate insecticides. Some pesticides were detected frequently at one location (acetochlor at Iowa) but not the other, as predicted based on crop and pesticide usage differences. Five compounds were detected only in 1995 or 2007. Voluntary removal of cyanazine from the US marketplace and its subsequent cancellation of registered uses in December 1999 resulted in a reduction from at least a 25% detection frequency at both sites in 1995 to no detections in 2007 samples.

**TP150 Derivation of a Site-specific Clean-up Level for Creosote-derived Polycyclic Aromatic Hydrocarbons** N. Bonnevie, ARCADIS, Risk Assessment and Ecological Services; J. Iannuzzi, D. Bax, P. Anderson, T. Iannuzzi, ARCADIS. There is increasing evidence that the assumptions used to derive default sediment benchmarks for polycyclic aromatic hydrocarbons (PAHs) are not applicable when evaluating the potential toxicity at sites where creosote-derived PAHs are expected to be the primary risk drivers. In particular, creosote-derived PAH in sediments appear to be less bioavailable than assumed by existing benchmarks. This is an important consideration when developing remedial goals for these sites as the standard screening benchmarks used to evaluate the potential toxicity of PAHs in sediments (e.g., Threshold Effect Level, Probable Effect Level, Threshold Effect Concentration, Median Effect Concentration, Extreme Effect Concentration, and the Equilibrium Partitioning Sediment Benchmark Toxic Unit [ESBTU]) may greatly overestimate the toxicity of creosote-derived PAHs to benthic invertebrates and possibly result in unnecessary and overly conservative remedial actions. This paper examines the toxicity of PAHs in sediments from multiple former wood-treating facilities using standard laboratory toxicity tests. Based on co-located sediment chemistry and toxicity data, the dose response curves indicated increasing toxicity with increasing PAH concentration. However, statistically significant toxicity did not occur until bulk sediment concentrations were much higher than would be predicted based on the currently available sediment benchmarks. In fact, there were samples with total PAH concentrations close to 200 mg/kg that exhibited little or no toxicity. The results of this investigation are consistent with previous evaluations suggesting site-specific clean-up levels for creosote-derived PAHs could range from 100 to more than 200 mg/kg.



**TP151 Ecological Risk Assessment of Oil Spill Affected Area: Visited After Two Years the *Hebei Spirit* Oil Spill, Korea** S. Hong, J. Khim, Korea Univ, Division of Environmental Science and Ecological Engineering; J. Ryu, Korea Ocean Research and Development Institute, Office of Policy Research; J. Park, S. Song, Korea Univ, Division of Environmental Science and Ecological Engineering; K. Choi, Seoul National Univ, School of Public Health, Seoul National Univ, Dept of Environ. Health; K. Ji, J. Seo, S. Lee, Seoul National Univ, School of Public Health; J. Park, W. Lee, Soonchunhyang Univ, College of Natural Sciences; Y. Choi, Citizens' Institute for Environmental Studies; K. Lee, C. Kim, NeoEnBiz Co, Institute of Environmental Protection and Safety; W. Shim, Korea Ocean Research and Development Institute; J. Naile, J. Giesy, Univ of Saskatchewan, Dept of Veterinary Biomedical Sciences and Toxicology Centre. In order to assess the ecological risk by residual crudes in coastal area after two years the *Hebei Spirit* oil spill (occurred in 2007), the crude-derived hydrocarbons, sediment toxicity, and benthic response were collectively investigated. Total of 50 surface and subsurface sediments from 22 locations along the coast of oil spill site were collected and measured for 1) hydrocarbon chemistry followed by 2) toxicity screening (H4IIE-luc bioassay), and 3) benthic macrofauna mapping (visual analysis). First, the concentrations of residual crude-derived hydrocarbons greatly varied among regions (or locations) and depth reflecting the impact of crude oils in site-specific manner. For example, the elevated residual crudes were mainly found in muddy bottom compared to sand sediments, particularly accumulated at subsurface layers. Some samples contained relatively great concentrations of several PAHs exceeding the corresponding ERL and ERM values. Looking for sediment toxicity, there was a good agreement between sedimentary concentration profiles and potential toxicity of organic extracts in concentration-dependent manner. A significant correlation ( $r > 0.73$ ,  $p < 0.01$ ) between log PAHs and dioxin-like activities also indicated a reasonable mass balance. However, highly weathered samples containing greater proportion of alkylated PAHs showed relatively greater dioxin-like activities indicating certain interaction under the modified composition of known and/or unknown Ah-R agonists from crude oils. Meanwhile benthic community response reflected somewhat confounding result that greater biodiversity and larger abundance in more contaminated sites, which suggested species-specific tolerance and/or recolonization of certain species (such as *Batillaria* spp.) during the weathering periods. All together, the site experienced a severe oil exposure seemed to be recovered to a certain degree after two years the *Hebei Spirit* oil spill occurred.

**TP152 PAHs Burden and Partitioning in Ten Biochar Matrices** A. Freddo, Univ of East Anglia; B. Reid, Univ of East Anglia, Environmental Sciences. When organic matter undergoes a pyrolysis process biochar is formed to obtain heat and power. During this process: the proportion of C increases by over 50% by weight with respect to the original feedstock; the structure of the matrix becomes porous and rich in biologically nutrients (e.g., potassium, phosphorous) and microelements (e.g., magnesium, calcium, manganese). Thus, it has been suggested that biochar can be amended to soil with a view to improving plant growth. Moreover, as a consequence of biochar's stable composition, the release of carbon (as CO<sub>2</sub>) back into the atmosphere is slow. Thus, there is the opportunity to sequestration of CO<sub>2</sub> into biomass (used as feedstock for biochar production) and then to store this carbon when biochar is used as a soil amendment. However, as pyrolysis products, biochars may contain significant levels of polycyclic aromatic hydrocarbons (PAHs). Amounts of these priority pollutants dependent upon feedstock type and the production processes (e.g., temperature). Establishing PAH concentrations and partitioning within biochar is essential to ensure safe incorporation of biochar in the environment. This study investigated exhaustive and non-exhaustive concentration of PAHs from ten complex carbonaceous matrices, produced from different starting material and under different pyrolysis temperature. We intend to present validation data for the extraction of PAHs from biochar to exemplify approaches that indicate PAHs partitioning and bio-availability. In light of these results, assessment of the risk represented by biochar and the risks associated with biochar incorporation into the soil will be evaluated, considering the toxic impact of biochar also in relation to the feedstock and pyrolysis temperature.

**TP153 A Toxicological Perspective on Alkylated PAHs as it Relates to Risk Assessment** A. Pawlisz, Conestoga-Rovers&Associates. Recent crude oil spills have reinvigorated the debate whether all polycyclic aromatic hydrocarbon (PAH) toxicity is accounted for in ecological and human health

risk assessments. PAHs are a mixture of hundreds of individual compounds and only a small fraction are routinely analyzed for, regulated, and assessed for harm to biological receptors. Increasingly, the alkylated homologs of PAHs are gaining attention in risk assessment due to their abundant constitution in fresh crude oils, relative persistence, bioaccumulation potential, not being quantified by common analytical methods, increasing relative proportion as crude oil ages, and potentially higher toxicity than the parent compounds. While one of the assessment methodologies is to consider homologue additive toxicity at par with their parent compounds, after applying an expanded scan for alkylated PAHs, there may be instances where this approach may still not accurately estimate the total PAH toxicity. A review of recent toxicological literature on alkylated PAH is provided with an insight on how to effectively account for added toxicity in an environmental risk assessment.

**TP154 Dermal Cancer Risks Associated with Exposure to Benzo[a]pyrene – of Potentially Greater Importance than Oral Exposures** A.L. Knafla, Equilibrium Environmental Inc. Humans are exposed to benzo[a]pyrene, a known carcinogen, at contaminated sites. An examination of rodent toxicity data indicates that on a per kilogram body weight basis, the skin may be a more sensitive organ following dermal exposure in comparison to other target tissues following ingestion exposure. Furthermore, traditionally human health risks from dermal contact with benzo[a]pyrene have been conducted by estimating the proportion of the dose that penetrates through exposed skin for comparison with an oral route Risk Specific Dose limit. However, as recently published by a group of investigators (Knafla et al. 2010), the development of epidermal tumours is a more sensitive endpoint than systemic tumours following dermal exposure. A mathematical algorithm was developed to allow for risk assessors to evaluate skin cancer risks following human exposures to benzo[a]pyrene in soil.

**TP155 Bioavailability Studies: The Last Available Tools For Evaluating PAH Risks Realistically** B. Magee, G. Hoeger, ARCADIS; B.J. Locoy, ARCADIS, Risk Assessment and Environmental Services; K. Connor, ARCADIS. Polycyclic aromatic hydrocarbon (PAH) risk assessment is currently overly conservative, with Regional Screening Levels that are orders-of-magnitude below anthropogenic background in most urban areas. Additionally, the United States Environmental Protection Agency (USEPA) is proposing to increase the Relative Potency Factors (RPFs) for 5 PAH and to increase the number of carcinogenic PAHs with RPFs from 6 to 26. Several proposed RPFs are 10x or greater including dibenz[a,h]anthracene (10x), benzo[c]fluorene (20x), dibenzo[a,l]pyrene (30x), and benz[j]aceanthrylene (60x). If this approach is adopted, human health risks from exposure to PAHs will increase considerably, and risk-based clean-up levels will drop to below background even for commercial/industrial receptors at the high end of the Superfund risk range. It is clear from the literature, however, that the mammalian bioavailability of PAH from weathered soils/sediments is considerably less than 100%. EPA and many State agencies have increasingly rejected the use of bioavailability adjustments derived from literature values. EPA has issued a recent report on the bioavailability of dioxins and furans in soils. In this report, the agency has supported a policy of performing in vivo bioavailability studies on site-specific media impacted by complex organic compounds. This paper will summarize literature bioavailability results for PAHs including a recent study performed by the authors on soils containing coal tar. Finally, the paper will discuss methodological issues regarding the planning and execution of a robust, internally consistent animal bioavailability study with site aged soils. Issues that will be discussed include type of controls (internal versus external), target PAHs, animal species and strain, sampled biological media (blood, urine, feces, other tissues), pharmacokinetic issues (single time point versus area under the curve), and analytes of interest (parent PAHs versus PAH metabolites vs DNA or protein adducts).

**TP156 An Analysis of Human Health Risks Arising from Exposure to PAHs Adjacent to Coal Tar Sealed Asphalt Parking Lots** E.S. Williams, Baylor Univ, Baylor Univ; B.J. Mahler, P.C. van Metre, United States Geologic Survey. Recent studies have demonstrated that concentrations of carcinogenic polycyclic aromatic hydrocarbons (PAHs) are higher in house dust in residences and soils adjacent to asphalt parking lots sealed with coal-tar based sealant (CSA) compared to the concentrations of PAHs in the same media adjacent to unsealed asphalt (UA) lots. Ten exposure scenarios were used to assess human health risks associated with ingestion exposure to

PAHs in soil and dust in CSA and UA settings. House dust PAH concentrations were from a study in Austin TX and soil PAH concentrations were from studies in Chicago IL and Durham NH. Maximum intake estimates for benzo[a]pyrene (BaP) in house dust and soil were considerably higher in exposure scenarios in CSA settings than in exposure scenarios in UA settings (55.6 and 1.79 ng BaP/kg/day, respectively). Excess lifetime cancer risk (ELCR) estimates associated with BaP and BaP equivalents (BaP<sub>EQ-7</sub>) were calculated using a cancer slope factor of 7.3 mg/kg/day<sup>-1</sup>. For lifetime incidental ingestion of house dust and soil in CSA settings, ELCR for BaP<sub>EQ-7</sub> is estimated at  $1.3 \times 10^{-4}$ , a 34-fold increase in risk relative to that for UA settings ( $3.8 \times 10^{-6}$ ). Estimated ELCR increases 11-12-fold in CSA settings relative to UA settings over the first 6 years of life because of higher ingestion rates and lower body weights. When ingestion of only house dust was considered, PAH exposures through incidental ingestion in CSA settings were estimated to increase ELCR 13-fold relative to UA settings, with 46% of that increase resulting from childhood exposure (ages 0-6) as a result of higher dust ingestion rates during childhood. Soil-mediated PAH exposures were estimated to elevate ELCR to a larger extent than house-dust-mediated ingestion, as PAH concentrations in soils in CSA settings are much higher than those in house dust and ingestion rates for soil are higher than those for house dust. On the basis of these results, we conclude that the use of coal-tar-based asphalt sealant products is associated with increased risk of cancer resulting from exposure to carcinogenic PAHs in soil and dust.

**TP157 Use of Mass Balance Bounding Estimates and Sensitivity Analysis to Prioritize PAH Inputs in Urban Systems** R.P. DeMott, T.D. Gauthier, Environ International.

PAH transport via the atmosphere and runoff inputs related to high density population and traffic in urban areas have been extensively studied for decades. In recent years, several studies focused on the use of specific PAH-containing materials, particularly pavement sealer products, as possible sources of sediment PAHs. The big-picture studies are advantageous for documenting patterns of PAH distribution actually established in the environment, but typically provide limited information about specific source inputs in a given location. Product-based studies can serve to identify locally relevant sources, but typically have limited resolution to evaluate relative inputs from numerous sources in larger systems. And, the types of controlled monitoring programs that can provide empirical information on the connections between specific sources and environmental load in an urban area are large, expensive and time-consuming. As a study design tool to help optimize monitoring programs, we first developed bounding estimates based on available literature for inputs that affect relative mass loading from long-range atmospheric, local combustion, traffic and pavement sealer sources of PAHs. We then used Monte Carlo-type simulations varying water body sizes, flow regimes and rainfall patterns, along with the ranges of values for the PAH input sources, to get a probabilistic sensitivity analysis. The output shows that critical factors include traffic density, rainfall washout patterns and the size of sealed parking lots relative to streams receiving uncontrolled runoff. These findings are useful in targeting streams and urban setting characteristics to monitor how municipal pavement sealer bans affect overall PAH exposure.

**TP158 Polycyclic Aromatic Hydrocarbons Analysis Using Chemical Mass Balance Model** C. Julias, CDM, CDM, Chemical Engineer; C. Liu, N. Luke, CDM. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic chemicals that persist in the environment. PAHs are formed through an incomplete combustion of most organic material and also occur naturally at low levels in crude oil and coal. Elevated concentrations of PAHs were found in soils in residential areas near an inactive chemical facility. The Chemical Mass Balance model (CMB-8.2) developed by the United States Environmental Protection Agency is used to identify the source(s) of anthropogenic PAH contamination in the residential areas. CMB model is a fundamental receptor model based on the use of the mass balance concept. Twelve parent PAHs are used in the CMB modeling because all 12 are frequently detected in soil samples and are included in many source profiles available in the literature. Concentrations of the 12 PAHs and total PAH for individual samples are used to generate PAH source profiles that are entered into the model. The CMB model consists of a least squares solution to a set of linear equations which expresses each receptor concentration of a chemical species as a linear sum of products of source profile species and source contributions. The model assumes that: (1) the composition of each source emission is consistent over the period represented by receptor data, (2) chemical species do not react with each other or with the environment, (3)

all sources that contribute significantly to the receptor have been identified and their chemical profile is known, (4) the composition of each source is linearly independent of other sources, and (5) measurement uncertainties are random, uncorrelated, and normally distributed. The CMB model attempts to derive source profiles from the covariation in space and/or time of many different samples of atmospheric constituents that originate in different sources. These profiles are then used in a CMB solution to quantify source contributions to each ambient sample. As a result, the CMB model is used to determine whether historical processes at the chemical facility that may relate to the deposition of contaminants in the residential areas.

**TP159 Managing Risks: Will Banning Pavement Sealers Have the Desired Effect?** K. O'Reilly, Exponent; J. Pietari, Exponent Inc.; P. Boehm, Exponent, Environmental & EcoScience Group, Exponent, Environmental Group, Exponent, Inc., Environmental & EcoScience sGroup. The USGS and others have raised the concern that refined tar pavement sealers are a significant source of polycyclic aromatic hydrocarbons (PAHs) in urban sediments. The researchers involved have been actively advocating product bans at the federal, state, and local level. While outlawing the use of an established product is one means of managing risks, it should be implemented only if it will have the desired effect. The USGS hypothesis is based in part on the similarity in PAH profiles between sealers and sediments. Our recent work has demonstrated that this profile is not unique, but common to a range of environmental particles that become entrained in sediments. Inconsistencies in the results of different forensic methods applied to the data used by the USGS further weaken their hypothesis. This presentation focuses on an evaluation of three urban water bodies, from the west coast, midwest, and east coast. Inclusion of these data in USGS publications has led to actual or proposed product bans. In each case, the water bodies are in air sheds that are part of atmospheric source studies. Forensic comparison of the PAH chemistry of the sediments, regional depositional particles, and sealers indicates that the sealers are not a controlling factor. The results suggest that implementing a sealer ban will not result in a change in local sediment chemistry. The implication of these findings in setting policies for risk management will also be discussed.

**TP160 Analytical and Relative Potency Factor Approach for Effective Evaluation of Polycyclic Aromatic Hydrocarbons for Risk Assessment** E. Krupka, S. Kirchner, V. Macwan, CDM.

Polycyclic aromatic hydrocarbons (PAHs) are chemicals that include organic compounds containing either two or more, or three or more, fused rings made up of hydrogen and carbon atoms. PAHs are persistent and primarily occur in the environment as complex mixtures produced from the incomplete combustion of substances containing hydrocarbons. Using a weight-of-evidence approach, the United States Environmental Protection Agency (EPA) has determined that 24 PAHs are carcinogenic based on toxicological similarities to the indicator compound benzo[a]pyrene. EPA has proposed using a relative potency factor (RPF) approach for estimating the cancer risk of PAH mixtures by summing doses of component PAHs using scaled doses of the individual PAHs relative to the potency of benzo[a]pyrene. Previously, RPFs were developed for seven carcinogenic PAHs by EPA in 1993. In this paper PAH analytical methods are compared for their potential effectiveness to yield useable data to evaluate all 24 carcinogenic PAHs necessary for the EPA's proposed RPF approach for use in risk assessment. The application of the methods for specific matrices, target compounds, quality control procedures, and reporting limits are evaluated and presented to add better understanding of analytical methods for data end users such as risk assessors. This information will assist in the selection of the appropriate method that can be applied to their study. Particular attention is paid to the method reporting limits and the analytical and sample matrix factors that affect data evaluation and application in risk assessments. Controlling the variables associated with method reporting limits is instrumental in enhancing the effectiveness of the data when applying derived results to the PAH mixture risk evaluation.

**TP161 Passive Dosing of Terrestrial Springtails – Linking Toxicity of PAHs and PAH Mixtures to Their Chemical Activity** S.N. Schmidt,

Aarhus Univ, NERI, Environmental Chemistry and Microbiology; M. Holmstrup, Aarhus Univ, NERI, Terrestrial Ecology; K.E. Smith, P. Mayer, Aarhus Univ, NERI, Environmental Chemistry and Microbiology. The chemical activity of organic soil contaminants has been proposed as an exposure parameter that quantifies the potential, direction and extent for physicochemical processes such as diffusion, partitioning and chemical

reactions. Thus, the partitioning of organic soil contaminants into soil living organisms is driven by their chemical activity. Passive dosing with the silicone poly(dimethylsiloxane) (PDMS) was used to control defined chemical activities of six polycyclic aromatic hydrocarbons (PAHs) and their mixtures in bioconcentration and toxicity tests with the terrestrial springtail *Folsomia candida*. The test animals could move freely on the PDMS loaded with PAHs, resulting in exposure through direct contact and saturated air. This presentation will summarize the results from bioconcentration and toxicity tests with single PAHs and their mixtures: (1) The uptake kinetics of naphthalene, anthracene, pyrene, benz(a)anthracene and benzo[a]pyrene were studied in bioconcentration experiments. The PAHs were extracted from springtails, and the extracts were analysed by high performance liquid chromatography with fluorescence detection. The results showed that stable equilibrium concentrations were reached for naphthalene, anthracene and pyrene within 7 days, and the three PAHs were therefore selected for two acute toxicity tests (7-days tests). (2) Naphthalene, phenanthrene (the toxic isomer of anthracene) and pyrene were tested at a wide range of chemical activities in order to establish chemical activity response relationships, and in order to determine effective chemical activities ( $\Sigma a$ ) for these three compounds. Single PAH lethality was successfully linked to chemical activity ( $a$ ), and the three  $\Sigma a$ -50's were all within a narrow range of chemical activity (0.019-0.052). (3) Finally, the springtails were exposed to 12 PAH mixtures with naphthalene, phenanthrene and pyrene at defined chemical activities in order to relate the lethality to the sum chemical activity ( $\Sigma a$ ) of the PAH mixtures. The toxicity data of all tested mixtures were successfully fitted to one chemical activity response relationship ( $r^2=0.89$ ), and the  $\Sigma a$ -50 of 0.027 was comparable to  $\Sigma a$ -50's for single PAHs. In summary, passive dosing was successfully applied to control the chemical activity of PAHs and their mixtures in bioconcentration and toxicity tests. The toxicity of 12 PAH mixtures could be fitted to a single activity response curve when using  $\Sigma a$  as a novel exposure parameter.

**TP162 Chlorinated Polycyclic Aromatic Hydrocarbons from the Combustion of Polyvinyl Chloride and Polyvinylidene Chloride** Y. Miyake, Univ of Shizuoka, Institute for Environmental Sciences; L. Tang, Univ of Shizuoka; Y. Fujimine, Otsuka Pharmaceutical Co. Ltd.; Y. Horii, Center for Environmental Science in Saitama; T. Amagai, Univ of Shizuoka. Chlorinated polycyclic aromatic hydrocarbons (ClPAHs) such as chlorobenz[a]anthracene and Chlorobenzo[a]pyrene have received worldwide attention because of their environmental persistence and widespread distribution. Recent reports have showed the occurrence of chlorinated or brominated polycyclic aromatic hydrocarbons in flue gas and fly ash from municipal and industrial waste incinerators. However, little is known about ClPAHs formation from combustion of chlorinated polymer such as polyvinyl chloride and polyvinylidene chloride alone or the influence of chlorinated polymer as a chlorine source in municipal and industrial waste incinerators. In this study, emissions evolved from the combustion of polyvinyl chloride and polyvinylidene chloride were studied between 500 to 1000°C in a cylindrical furnace with a diameter of 28 mm and a length of 405 mm, in order to analyse the influence of both temperature and materials on the by-products such as ClPAHs. Twenty individual ClPAHs, representing mono- through trichloroPAHs, were determined to be from the following: chlorofluorene (ClFle), chlorophenanthrene (ClPhe), chloroanthracene (ClAnt), chlorofluoranthene (ClFlu), chloropyrene (ClPyr), chlorochrysene (ClChr), chlorobenz[a]anthracene (ClBaA), and chlorobenzo[a]pyrene (ClBaP).

**TP163 Effects of Bioremediation on Toxicity and Genotoxicity of PAH-contaminated Soil Using Genetically Engineered DT40 Cell Lines** J. Hu, J. Nakamura, S.D. Richardson, M.D. Aitken, Univ of North Carolina at Chapel Hill, Dept of Environmental Sciences & Engineering. Bioremediation is one of the commonly applied remediation strategies at sites contaminated with polycyclic aromatic hydrocarbons (PAHs). However, it remains controversial whether bioremediation could reduce health risks while removing the target compounds. This study investigated changes in the toxicity and genotoxicity of PAH-contaminated soil from a former manufactured-gas plant site before and after two simulated bioremediation processes: treatment in a laboratory sequencing batch reactor system and a long-term, continuous-flow column system. Toxicity and genotoxicity of the residues from soil extracts were determined by multi-well plate-based DNA damage response analysis using the chicken DT40 B-lymphocyte isogenic cell line and its DNA-repair deficient mutant cell lines. The results demonstrated that both bioremediation processes can significantly remove PAHs

from the contaminated soil (bioreactor 52% removal; biostimulated column 63% removal). However, the bioreactor treatment resulted in an increase of toxicity and genotoxicity, whereas long-term column treatment resulted in a decrease of toxicity and genotoxicity. The  $LD_{50}$  for DT40 and the RAD54 mutant decreased from  $1.31 \pm 0.08 \mu\text{g/mL}$  and  $0.53 \pm 0.10 \mu\text{g/mL}$ , respectively, to  $0.60 \pm 0.03 \mu\text{g/mL}$  and  $0.12 \pm 0.02 \mu\text{g/mL}$ , respectively in the bioreactor. The  $LD_{50}$  for DT40 and RAD54 increased from  $2.36 \pm 0.17 \mu\text{g/mL}$  and  $1.25 \pm 0.01 \mu\text{g/mL}$  to  $8.61 \pm 0.17 \mu\text{g/mL}$  and  $3.48 \pm 0.10 \mu\text{g/mL}$ , respectively in the biostimulated column. We also found that when controlling  $1/C_{IPAHs}$ , the partial correlation between  $LD_{50}$  values and  $1/C_{residue}$  was statistically significant ( $p < 0.01$ ); but when controlling  $1/C_{residue}$ , the partial correlation between  $LD_{50}$  values and  $1/C_{IPAHs}$  was not statistically significant. Furthermore, when screening the column soil with a battery of DT40 mutants for genotoxicity, the results showed that mutant XPA was not sensitive to either untreated soil or treated soils; mutants RAD9, RAD17, MSH2, RAD54, RAD18, PCNA, REV1 and POLQ were sensitive to both untreated and treated soils; mutants POLB, FEN1, KU70, REV3, POLK and POLH were only sensitive to treated soils but not to untreated soil. These results suggest that bioremediation does not necessarily reduce the toxicity and/or genotoxicity of PAH-contaminated soil, and that different bioremediation processes can have different effects on toxicity or genotoxicity.

**TP164 PAH Body Residues and Lysosomal Membrane Destabilization in Mussels Impacted by T/V Dubai Star Oil Spill** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering, Univ of California, Dept of Civil and Environmental Engineering; B. Stanton, California Dept of Fish and Game; T. McBride, US Fish and Wildlife Service; M. Anderson, California Dept of Fish and Game. A fuel oil spill from the *T/V Dubai Star* occurred in San Francisco Bay on October 30, 2009. It is important to assess the impacts of spilled oil on living organisms in a timely manner considering that evidence such as body residues and biological responses can diminish over time as the levels of various toxic chemicals in the water column decline by dilution and natural weathering. Mussels were collected over three months from the eastern shorelines of the central bay for the measurement of PAHs and lysosomal destabilization. PAH body burdens increased sharply and declined exponentially during 90 days of the study period. Environmental half-life of PAH body residues was 16 days. PAH body residues were above critical body residue (2,100 ng/g dry wt.) in some highly impacted sites. Lysosomal destabilization exhibited a positive correlation with PAH body residues. Though sample size is small, this study demonstrates that lysosomal destabilization could be used successfully as a rapid, cost-effective screening tool to identify exposure to and potential injury by spilled oil as well as help guide further possible damage assessment studies.

**TP165 Classifying Environmental Hazards under CLP – Incorporating Science and Best Practices** J. Small, Merck & Co., Inc., Global Safety and the Environment, Merck & Co., Inc.; G. Gagliano, L. Ziv, J. Tell, K. Silverman, Merck & Co., Inc., Global Safety & the Environment. On January 20, 2009, Regulation (EC) No 1272/2008 on the classification, labeling and packaging of substances and mixtures (CLP), replaced the Dangerous Substance Directive 67/548/EEC and the Dangerous Preparations Directive 1999/45/EC. CLP is part of the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals in the European Union (EU) and is aligned with EU legislation already in existence. The system is designed to facilitate international trade of chemicals and to maintain existing levels of protection for human health and the environment. One of its main intentions was to introduce consistency in how chemicals are classified and labeled. However, our initial experiences have revealed potential shortcomings in the guidance that could result in misinterpretation, and a final result that is not harmonized. These inconsistencies in classification can have implications for downstream users (customs officials, shipping/transportation Depts, environmental scientists, first responders) unfamiliar with the parameters used for the classification assessment. The environmental classification system consists of five categories for labeling, and includes one acute toxicity category and three levels of chronic toxicity based on effect and/or lethal concentration values of various aquatic indicator species. The fifth category, labeled Chronic Aquatic Hazard Category 4, is known as the "safety net classification." This category was created to help classify compounds with ambiguous toxicity that may be a potential for concern. The guidelines outline certain parameters that places a compound in this category; however, they are not exhaustive and leave room for interpretation and professional judgement. Our experience has identified key decision points to consider



when classifying chemicals in order to reduce ambiguity and increase overall consistency among risk assessors. Critical to these decisions are consideration of more than just the acute toxicity to aquatic organisms, the octanol/water partition coefficient (K<sub>ow</sub>) and/or bioconcentration factor (BCF) of a compound. We suggest also including environmental fate factors such as water solubility, ionization and degradability during classification assessments. Examples and lessons learned are presented.

**TP166 ERA and Human Medicinal Product Marketing Authorization: Framework for an Optimized Risk Information Strategy** L. Meisel, INFARMED; A. Pena, CEF, Faculty of Pharmacy, Univ of Coimbra, Coimbra, Helath Surveillance Group; N. Adler, UBA Umweltbundesamt. EU environmental policy is based on the precautionary principle and state that preventive action should be taken. Thus, in accordance with Article 8(3) of Directive 2001/83/EC the environmental risk for Human Medicinal Products should be assessed. However, the evaluation outcomes does not constitute criterion for a Marketing Authorisation refusal. In this case, an understanding of risk and the application of risk assessment methodology is essential to being able to efficiently and effectively create minimisation, safety measures which will be communicate in product labelling. Unfortunately limited data, essentially for "older" Human Medicinal Products make difficult to determine the likelihood of the risk and possible impact values. Generally a standard statement is included in package leaflet as consumer information to appropriate disposal of unused pharmaceuticals. Moving to a more science-based ecological risk communication for the pharmaceuticals we developed a framework of an Optimized Risk Information Strategy (*what, whom and how*) for the Summary of Products Characteristics (health professionals) as well as for Package Leaflet (consumers, public). As such, efforts will be made towards to collect valid information: (1) from quantitative or qualitative risk assessment; (2) from minimization measures considering particular vulnerabilities which apply to the different pharmaceutical groups e.g., cytostatic, antibiotics; (3) from fundamental precept information supporting, for instance, occurrence, consumption in various EU member states. This strategy should comprise a part of an overall risk communication approach agreed upon outside manager parties to the information dissemination, during the use, waste phase of Human Medicinal Products.

**TP167 Evaluation of a Method for Conducting OECD Testing for Transformation of a Material in an Aquatic Sediment System and Toxicity to a Benthic Organism** C. Picard, Smithers Viscient, Sediment Ecotoxicology; S. McLaughlin, K. Malekani, Smithers Viscient, Environmental Fate and Metabolism; M.J. Bradley, Smithers Viscient, Sediment Ecotoxicology. The current European Medicines Agency risk assessment guidance calls for investigation of toxicity to sediment dwelling midge larvae (OECD 218 guideline) when greater than 10% of the test material partitions to the sediment at day 14 or later in sediment/water systems under aerobic conditions (OECD 308 guideline). The typical maximum exposure duration in the OECD 218 testing is 28 days. While this guidance may provide information on the toxicity of a parent compound to a sediment dwelling organism, it does not address the effects of a test material to sediment dwelling organisms over longer durations that may be encountered in the environment. Material may degrade in the sediment or bind tightly over longer periods of time potentially affecting the toxicity to sediment dwelling organisms. In this hybrid study design we attempt to achieve the following: (1) investigate the biological performance of midge larvae (*Chironomus riparius*) in two natural sediments historically used in OECD 308 testing and compare to performance in OECD formulated sediment. (2) characterize the microbial biomass in these natural sediments as well as the formulated sediment at various time points in order to compare as it pertains to degradation rates. (3) develop and validate a test method that investigates the transformation of a known test material applied to the water in the various sediment/water test systems over 100 days while synchronously exposing multiple populations of midge to the changing test system throughout the duration of the test. The typical biological endpoints of percent emergence and development rate will be measured on each midge population throughout the various phases of the study. This test method will theoretically provide information on the transformation of a test material in various sediment/water systems over time while also providing toxicity information as it regards to the parent compound as well as any metabolites that are produced throughout the 100 day test period. This test method could be used as a possible supplement to the typical OECD 308 and 218 guidelines.

**TP168 Life-Cycle Exposure of *Daphnia magna* to Environmentally Relative Mixtures of Pharmaceuticals** D.N. Wolfe, S. Richards, Univ of Tennessee at Chattanooga; M. Hanson, Univ of Manitoba. Due to the global detection of pharmaceuticals in surface water, risk assessments require toxicity testing to be performed to close knowledge gaps for effects assessment regarding impacts on aquatic food web dynamics. The widespread use of pharmaceuticals has resulted in mixture concentrations of mg/l in effluent and ug/L concentrations in surface water. Their potential toxicological effect on fresh water ecosystems remains largely unknown, especially as complex mixtures. Toxicity data on the effect of pharmaceuticals has expanded in the past decade, but has mainly focused on single, acute pharmaceutical exposures, not environmentally realistic mixtures. By determining the threshold of response for environmentally relevant mixtures, the risk that these compounds may pose to the environment will be determined more accurately. In 2008, thirteen pharmaceuticals were quantified in the Tennessee River, USA and its tributaries, ranging from 0.1757 to 0.0028 ug/l. Using the same ratios of individual compounds, but increased concentrations, the present study conducted chronic life cycle toxicity test on the cladoceran *Daphnia magna*. Tests were performed using 10x, 100x and 1000x the concentrations detected in the Tennessee River, resulting in a total concentration of 6.031, 60.31, and 603.1 ug/l of total pharmaceutical exposure, respectfully. Mortality, time to first brood, size and fecundity were used as endpoints of toxicity. Test solutions were renewed three times a week and neonate removed each day until the conclusion of the experiment. *D. magna* showed a statistically significant decrease in the number of neonate produced when exposed to 100x and 1000x ( $p < 0.003$ ). When *D. magna* was exposed to the 10x concentration, no significant decrease in neonate production was observed. Neither time to first brood nor size was affected at 10x, 100x, or 1000x. Studies are on going to pinpoint the threshold of effects occurring between 10x and 100x. These data will provide risk assessors more accurate data when analyzing non-lethal effects of pharmaceutical mixtures on aquatic ecosystems.

**TP169 PhATE Model for Predicting Concentrations of Trace Organics in Sludges and Biosolids from Municipal Wastewater Treatment Plants (WWTPs)** V.L. Cunningham, Sustainability Sciences LLC; V.J. D'Aco, Quantum Management Group, Inc.; D. Pfeiffer, Arcadis US, Inc.; P. Anderson, ARCADIS; M. Buzby, Merck & Co., Inc.; R.E. Hannah, GlaxoSmithKline; J.J. Jahnke, Merck & Co., Inc.; N.J. Parke, Eli Lilly and Company. PhATE™, now expanded to include sludges and biosolids, was originally developed to estimate potential concentrations of active pharmaceutical ingredients (APIs) in US surface and drinking waters that could result from patient use of medicines. The model estimates the mass of API in WWTP influent based on the population served, the API per capita use, and the potential loss of the compound associated with human use (e.g., metabolism). The mass of API on the treated biosolids is then estimated based on partitioning to primary and secondary solids, potential loss due to biodegradation in secondary treatment (e.g., activated sludge), and potential loss during sludge treatment (e.g., aerobic digestion, anaerobic digestion, composting, etc.). Simulations using two surrogate compounds show that predicted environmental concentrations (PECs) generated by PhATE are in very good agreement with measured concentrations, i.e., well within one order of magnitude. Model simulations were then carried out for 18 APIs representing a broad range of chemical and use characteristics. These simulations yielded four categories of results: (1) PECs are consistent with measured data for nine compounds with high analytical detection frequencies; (2) PECs are greater than measured data for three compounds with high analytical detection frequencies, possibly as a result of as yet unidentified depletion mechanisms; (3) PECs are less than analytical reporting limits for five compounds with low analytical detection frequencies; (4) the PEC is greater than the analytical method reporting limit for one compound with a low analytical detection frequency, possibly again as a result of insufficient depletion data. Overall, these results demonstrate that because of the concordance between measured concentrations and those predicted by PhATE, it has the potential to be a very useful tool in the evaluation of APIs in biosolids. Possible applications include: prioritizing APIs for assessment even in the absence of analytical methods; evaluating sludge processing scenarios to explore potential mitigation approaches; and developing realistic nationwide concentrations, since PECs can be represented as a cumulative probability distribution. Finally, comparison of PECs to measured concentrations can also be used to identify the need for fate studies of compounds of interest in biosolids.

**TP170 Mercury Concentrations in Fillets of Fish Collected in the USEPA National Rivers and Streams Assessment of the Continental USA** J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; E. OByran, J. Webb-Turbeville, Dynamac Corp c/o USEPA Office of Research and Development, National Exposure Research Laboratory; M. Crane, A.L. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; L.L. Stahl, J.B. Wathen, USEPA Office of Water, Office of Science and Technology. The National Rivers and Streams Assessment (NRSA) is a statistical survey of flowing waters of the US. The purpose of this survey was to assess the condition of the Nation's rivers and streams, establish a baseline to evaluate progress of pollution control activities in flowing waters of the US, and generate statistically valid and environmentally relevant reports on the condition of the Nation's water resources. The survey measured a wide variety of variables intended to characterize chemical, physical, and biological condition. These variables included nutrients, chlorophyll-a, other water chemistry analytes, sediment enzymes, enterococci, fish tissue, physical habitat, and biological characteristics, including assessments of phytoplankton, periphyton, benthic macroinvertebrates, and fish communities. In 2008 and 2009, fish were collected from about 650 streams and rivers, including a representative subset of 183 urban river sites. The USEPA analyzed fish fillets for 22 pesticides, 20 congeners of PCBs, 8 congeners of PBDEs, mercury, selenium, % lipids and % moisture. Mercury was detected in all samples and ranged from 14.8 to 1418.54 ng/g in the fish fillet samples, an unaverage concentration across all samples of 230.91 ng/g and a median of 174.95 ng/g. Nearly 26% of the samples exceeded the EPA 300 ng/g screening value for recreational fishing and about 98% of the samples exceed a wildlife screening value of 30 ng/g. A statistical analyses and assessment from a national scale will be presented.

**TP171 Comparison of Mercury and Selenium Concentrations in Whole Fish and Fillets Analyzed in Fish Collected in the Lower Mississippi River** M. Crane, USEPA Office of Research and Development, National Exposure Research Laboratory; E. OByran, J. Webb-Turbeville, Dynamac Corp c/o USEPA Office of Research and Development, National Exposure Research Laboratory; A. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; T. Angradi, D. Bolgrien, USEPA Office of Research and Development, National Health and Environmental Effects Research Laboratory; L.L. Stahl, USEPA, Office of Water, Office of Science and Technology, Office of Science and Technology. The science and practice of environmental assessment have been advanced by research done by the EPA Office of Research and Development's (ORD) Environmental Monitoring and Assessment Program (EMAP). In short, the condition of a resource can be estimated from key chemical, physical, and biological measurements at relatively few sites. It is analogous to a public opinion survey where the responses of a few people provide estimates for the entire population. EMAP's statistical sampling designs and biological indicators are well developed. The Office of Water (OW) has adopted them for its national assessments of aquatic resources. As part of the USEPA National River and Streams Assessment (NRSA) and EMAP Great Rivers Projects, 13 urban sites and 43 non urban sites on the lower Mississippi River were sampled for fish to be analyzed for mercury and selenium in whole fish and fillets. In a previous EMAP GRE study, the upper Mississippi River was sampled but only whole fish samples were analyzed for Hg. EMAP-GRE focused on whole fish because of its emphasis on the health of the ecosystem. Although whole-fish contamination is primarily an indicator of risk to piscivorous wildlife, whole fish data are still relevant for estimating human risk from fish consumption. At each of the 56 sites, three fish samples were collected: a primary, a secondary sample and a sample for fillets. A comparison was made between whole fish secondary samples and fillets. Preliminary analyses indicates that whole fish levels of Hg were found to be from 19% less to 30% higher than fillets and selenium was found to be 13% less and 42% higher than fillets. A statistical analyses and assessment of whole fish and fillets concentrations vs. wildlife and human risk of consumption will be presented.

**TP172 Form of Methylmercury Present in Diet Does Not Affect Bioaccumulation in Various Tissues in the Zebra Finch (*Taeniopygia guttata*)**

C.W. Ramos, M. Whitney, J.P. Swaddle, D.A. Cristol, College of William and Mary. Most methylmercury in living organisms is found bound to the amino acid cysteine. In this form it can be readily taken up by tissues, incorporated into proteins, and is even actively transported across the blood brain barrier. Although methylmercury-cysteine is the most common form of mercury in natural food webs, most methylmercury dosing experiments use methylmercury-chloride, a commercially available salt. It is unknown whether these compounds may behave differently within an organism. In this study we investigate whether the form of methylmercury presented in the diet affects bioaccumulation of mercury in various tissues in the Zebra Finch (*Taeniopygia guttata*), particularly focusing on whether the more natural form, methylmercury-cysteine, may more easily pass into the brain or into the egg. Birds were maintained on a diet containing either methylmercury-cysteine or methylmercury-chloride at one of two levels (0.5 ppm or 1.0 ppm) for 10 weeks until blood mercury levels stabilized. The birds were then allowed to breed and eggs were collected for mercury analysis. After approximately 25 weeks, the birds were humanely sacrificed and mercury levels were measured in the blood, brain, liver, kidney and muscle. There was no difference in the amount of bioaccumulation of mercury between the two forms in any of the tissues measured. It is possible that because of methylmercury's high affinity for cysteine, it becomes quickly associated with cysteine during the digestion process. These results suggest that the form of mercury used in dietary dosing experiments has little effect on levels of mercury bioaccumulation in tissues throughout an organism.

**TP173 Study Design and DQO for Development of Plant and Invertebrate Preliminary Remedial Goals for the US Dept of Energy Hanford Site** C. McCarthy, CH2M Hill, Environmental Services; B. Sample, Ecological Risk, Inc.; J. Lowe, CH2M HILL Plateau Remediation Company; A. Aly, Interra; J. Hansen, United States Dept of Energy. The US Dept of Energy, Hanford Site has soil concentrations of non-radioactive chemicals that exceed the State of Washington and EPA plant and invertebrate screening levels. However, these screening levels may not reflect Hanford Site specific conditions. A study was designed to: (1) perform plant and soil invertebrate bioassays on field-collected soils, 2) perform detailed soil chemistry analyses on these samples, and (3) integrate the chemistry and effects data to identify analyte specific thresholds for effects reflective of the Hanford Site. A three-step process was employed to identify sample locations representing a range of soil analytical chemistry and geochemistry present throughout the site: 1) low, medium, and high priority analytes were identified; 2) low, medium, and high target concentration ranges were established for priority analytes through comparison of site data to existing screening values, background, and cleanup goals; and 3) historic data were reviewed to identify possible sampling locations with contaminant levels within the target concentration ranges. To optimize the number of bioassay samples within established target concentration ranges, the analytical chemistry and bioassay samples were collected in one mobilization with the bioassay samples being archived until the chemical analysis was complete. Analytical chemistry and geochemical data (pH, TOC, CEC, grain size) were then reviewed to select a subset of samples for bioassays. Bioassays were not performed on samples with detections of pesticides or herbicides or field controls with measured concentrations above background. The selected bioassays included Sandberg bluegrass (*Poa secunda*) 14 day post germination and 28 day growth bioassays and springtail (*Folsomia candida*) 28-day reproduction and survival bioassays. These tests were selected for their broad applicability to all terrestrial environments found at the Hanford Site. The study design's analytical approach calls for: establishing significance of observed effects relative to field controls; analysis of paired chemical, geochemical and bioassay data to identify dose-response relationships, linear regression analysis, establishment of NOECs, LOECs, 20 and 50 percent effects levels; and multivariate analysis as warranted.

**TP174 Ecotoxicological Assessment of Arsenic and Lead Contaminated Soils in Former Orchards at the Hanford Site** D. Delistraty, Washington State Dept of Ecology, Washington State Dept of Ecology; J. Yokel, Washington State Dept of Ecology. The purpose of this study was to assess ecotoxicity of former orchard soils contaminated with lead arsenate pesticides at the Hanford Site in Washington state (USA). Surface soil, plant, and invertebrate samples were collected from 11 sites in former orchard areas near the 100-H Area during May and August 2010. Mean (standard deviation [SD]) As and Pb concentrations in soil were 39.5 (40.6) and 208 (142) mg/kg dry weight (dw), respectively (n=11). As and Pb concentrations



in surface soils were positively and significantly correlated ( $r=0.87$ ,  $P<0.05$ ). Speciation of total inorganic As in soil ( $n=6$ ) demonstrated that mean As+5 was the dominant form (>99%), whereas mean As+3 was minor (<1%). Mean (SD) As and Pb in cheatgrass were 3.91 (7.89) and 12.4 (20.0) mg/kg dw, respectively ( $n=11$ ), while mean (SD) As and Pb in darkling beetles were 5.37 (2.64) and 3.87 (2.95) mg/kg dw, respectively ( $n=8$ ). Simple linear regressions were constructed to estimate soil to plant uptake for As and Pb, as well as soil to invertebrate uptake for As and Pb. Regressions were significant ( $P<0.05$ ) only for soil vs. plant (As) and soil vs. invertebrate (Pb). Standardized lettuce seedling and earthworm bioassays were performed with a subset of soil samples ( $n=6$ ). No significant effects ( $P>0.05$ ) were observed in lettuce survival or growth nor in earthworm survival or sublethal effects, relative to negative laboratory controls. In particular, metallothionein (a protein biomarker for metal exposure) in these earthworms showed no significant difference ( $P>0.05$ ), relative to its control. Based on these bioassays, unbounded NOECs in soil for As and Pb were 128 and 390 mg/kg dw, respectively, representing the maximum soil concentrations observed in our study. These NOECs are applicable only to former orchard soils at Hanford (due to limited soil sampling), and their associated uncertainty relates in part to an incomplete dose-response relationship with non site-specific test organisms. Data from the literature demonstrated that mean As and Pb soil concentrations exceeded Hanford background levels but were similar to other orchard soils. Furthermore, although mean As and Pb soil concentrations were below bioassay-derived NOECs in this study, mean soil concentrations were in the range of ecotoxicological benchmarks reported in the literature.

#### TP175 Pentachloronitrobenzene/Quintozone in the Great Lakes L.M.

Jantunen, Environment Canada; J. Struger, Environment Canada, Water Science and Technology Directorate; K. Su, K. Brice, Environment Canada; H. Hung, Environment Canada, Science and Technology; S. Backus, Environment Canada. Pentachloronitrobenzene (PCNB), the active ingredient in quintozone, is a fungicide de-registered for usage in Canada and the USA. In December 2010, although existing stocks can be used through December 2011. Its main usage is to control mould on golf courses. In contrast to other pesticides, quintozone is applied to golf courses in late fall and early spring. Due to its solubility and vapour pressure, it is expected to be found in air and water around the Great Lakes. Thirteen Lake Ontario tributaries were sampled monthly starting in October 2010 and three Lake Huron tributaries were added in May 2011. The highest level was found at Spencer Creek in December 2010 at 187 ng/L, this creek runs through a golf course. Other water samples sites showed spikes in the late fall/early winter where some sites showed spikes in February/March. Additionally, the Integrated Atmospheric Deposition Network (IADN) investigated PCNB in air at three Canadian sites from January 2004 to the present. The sites were in rural areas: Point Petre is on the north shore of Lake Ontario, Burnt Island is in northeastern Lake Huron and Egbert is an agricultural site not situated on a lake. Egbert had the highest median air concentration of PCNB (32 pg/m<sup>3</sup>), followed by Point Petre (14 pg/m<sup>3</sup>) and the lowest levels were seen at Burnt Island (6.8 pg/m<sup>3</sup>). As with the water measurements, PCNB also showed seasonality, with the lowest levels in June to October at all three sites. Levels were highly variable during other times of the year, showing spikes with a maximum concentration in November 2006 of 2.9 ng/m<sup>3</sup> at Egbert.

#### TP176 Acute Toxicity of Five Fungicides to Select Freshwater Invertebrates J.L. Kunz, USGS; C. Ingersoll, USGS, Columbia Environmental Research Center; R.D. Calfee, USGS, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; E. Little, USGS, Columbia Environmental Research Center; K. Kuivila, K.L. Smalling, US Geological Survey. The objective of this study is to conduct a series of acute toxicity range-finding tests using five fungicides and select freshwater aquatic invertebrates, given the limited availability of toxicity data for invertebrates other than cladocerans. Results of these studies will be used to identify species and fungicides that might be evaluated in more definitive acute toxicity tests or in chronic water-only or chronic spiked-sediment toxicity tests. Given the modes of action through which fungicides exert toxic effects (e.g., disruption of mitosis, suppressed respiration, altered lipid and protein synthesis), these chemicals will likely exhibit toxicity to a broad range of taxa. Five fungicides with unique modes of action, included in parentheses, are being evaluated: (1) azoxystrobin (respiration); (2) boscalid (respiration) (3) myclobutanil (sterol biosynthesis in membranes); (4) iprodione (signal transduction/osmoregulation/oxidative damage); and,

(5) propiconazole (sterol biosynthesis in membranes, demethylase inhibition (CYP450)). The high concentrations (10x reported LC50s for cladocerans) for the 5 fungicides of interest ranged from 259 to 110,000 ug/L. The test organisms being evaluated include: (1) Cladocerans (*Ceriodaphnia dubia*, *Daphnia magna*); (2) Amphipods (*Hyalella azteca*); (3) Mayflies (*Hexagenia* spp.); (4) Midge (*Chironomus dilutus*, *C. riparius*); (5) Mollusks (mussels, *Lampsilis siliquoidea*; snails *Lymnaea stagnalis*); and, (6) Oligochaetes (*Lumbriculus variegatus*). Endpoints being evaluated include: (1) 48-h survival after exposure to select fungicides spiked in water and (2) 4-h survival after a subsequent pulse exposure to 25  $\mu$ W/cm<sup>2</sup> UV lighting to assess potential photo-enhanced toxicity of UV to exposed invertebrates.

#### TP177 Assessment of Aerial Fungicide Application Drift and Toxicity to Freshwater Organisms B. Holzer, Oklahoma State Univ, Dept of Zoology, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology; J.B. Belden, Oklahoma State Univ, Dept of Zoology. Previous studies indicate that strobilurin fungicides are toxic to freshwater fish and invertebrates, as well as amphibians. The foliar fungicide Quilt Xcel™, which contains the active ingredients propiconazole (triazole fungicide) and azoxystrobin (strobilurin fungicide), is applied one to two times per growing season to prevent disease in cereal crops and legume vegetables. Because this fungicide is often applied aerially, there is potential for spray drift to contaminate adjacent untreated fields as well as nearby aquatic systems. Additionally, if the fungicides reach the ground following application, they may subsequently enter aquatic systems in runoff. Although propiconazole and azoxystrobin have been shown to cause low to moderate toxicity (respectively) to freshwater aquatic organisms, very little information is available concerning chronic and joint toxicity. Furthermore, limited information is available for nontarget fungi. The objectives of this study were to: monitor fungicide application and potential aerial drift to corn and soybeans at varying vertical locations from plant canopy to soil in both treated and adjacent untreated fields; assess the effect of the fungicides on soil fungal activity; assess the potential of aerially applied fungicides to contaminate nearby aquatic systems in a post-application runoff event; and assess toxicity of propiconazole and azoxystrobin individually and as a mixture to freshwater aquatic organisms including *Hyalella azteca*, *Daphnia pulex*, *Danio rerio*, and *Pimephales promelas* using chronic endpoints.

#### TP178 Acute Toxicity of Three Strobilurin Fungicide Formulations and Their Active Ingredients in Tadpoles E.A. Hooser, S.T. McMurtry, J.B. Belden, Oklahoma State Univ, Dept of Zoology; L.M. Smith, Oklahoma State Univ. In the past decade, field applications of strobilurin fungicides has sharply increased due to disease outbreaks and proclaimed plant yield benefits. Although not labeled for aquatic systems, spray drift, overspray, and runoff during storm events can result in pesticide input in adjacent non-crop areas, including wetlands embedded in agricultural fields. Active ingredients (AIs) in some fungicide formulations have shown acute toxicity to selected aquatic organisms at environmentally relevant concentrations. In addition, a recent study demonstrated acute toxicity of formulations to amphibian larvae at and below environmentally relevant concentrations. However, there is a lack of comparable data for toxicity between AIs and formulations. Adjuvant chemicals, which may have toxic effects separate from AIs, are the majority component of formulations. The mode of action for strobilurins is not specific to fungi as they bind to mitochondria and inhibit cellular respiration and ATP acquisition, thus there is potential for having deleterious effects on non-target species. However, adjuvant chemicals have an unrelated mode of action specific to the formulation, which may also cause toxicity. Expanding upon previous investigations, acute toxicity of Headline®, Stratego®, and Quilt® and their AIs (pyraclostrobin, trifloxystrobin & propiconazole, and azoxystrobin & propiconazole, respectively) were tested separately using mortality as the endpoint. Comparisons of mortality responses were made using Great Plains toads, *Bufo cognatus*, as the model species because of its high probability of being exposed and its ability to be a good surrogate for other *Bufo* species. Tadpoles were exposed to four concentrations of fungicides for 96 hours to assess the dose-response relationship, to determine 96hr-LC<sub>50</sub> values, and to compare the toxicity of AIs and formulations at multiple concentrations. Mortality responses were recorded after 24, 48, 72, and 96 hours. This experiment will help further refine our understanding of toxicity responses observed in previous studies



on Great Plains toads by elucidating the role of AIs versus other formulation ingredients.

**TP179 Asymmetric Patterns of Cranial Skeleton of Zebrafish Induced After Exposure to NaPCP in different Embryonic Developmental Stages** F. Lopez-Romero, Escuela Nacional de Ciencias Biológicas-I.P.N.; G. Zuniga-Bermudez, Instituto Politecnico Nacional, Escuela Nacional de Ciencias Biológicas; F. Martinez-Jeronimo, Escuela Nacional de Ciencias Biológicas-I.P.N., Zoology, E.N.C.B.-I.P.N. Organisms with bilaterally symmetry produce a reflected morphological copy in both sides of their body, which develops under control and regulation of the same gene array. Environmental factors influence the phenotype and the exposure to teratogenic pollutants produce disruption in development when a specific developmental stage is exposed. Sublethal toxicity tests were conducted at different developmental stages (zygote-blastula, blastula-gastrulation, gastrulation-segmentation, segmentation-pharyngula, and pharyngula-larva) of zebrafish (*Danio rerio*), exposing to the non-observable effect concentration (NOEC) of the pesticide Sodium Pentachlorophenate (NaPCP), to find susceptibility windows. Shape variation in the viscerocranium induced by NaPCP was estimated as Fluctuating Asymmetry (FA) (which is thought to increase with environmental stress), using geometric morphometric techniques, which eliminate differences in size and evaluates only changes in shape. A Procrustes ANOVA was done from a symmetric consensus, measuring variation around this consensus for the following factors: Individual variation in shape (Individuals), side variation (Sides), Individuals x Sides interaction (FA), and the effect for exposure in the developmental stage (Stage). Individual variation, Stage and FA were the factors which accounts for most of the variation ( $p < 0.0001$ ). The exposure window comprising Segmentation to Pharyngula exhibited more deformities among all the structures in viscerocranium. Results suggest that NaPCP might disrupt signaling pathways involved in the development of cranial skeleton and osteogenesis.

**TP180 Biomarkers to Access Metal Exposure in Estuarine Fish** A. Machado, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas; J. Cardozo, E. Gomes, M. Muller Hoff, Universidade Federal do Rio Grande – FURG; A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. Several human activities have contributed to increase copper input into aquatic environments. In order to access aquatic health, environmental regulations usually establish the water quality criteria for copper based on its concentration in the water. However, biological impact associated with copper contamination usually cannot be inferred from this parameter. In this context, biomarkers can be useful tools to access potential impacts. However, there is a lack of information on biomarkers in tropical estuarine fish exposed to environmentally relevant concentrations of copper. In the present study a set of biomarkers were evaluated in the fish *Poecilia vivipara* exposed to waterborne copper (0, 5, 9 and 20 µg Cu/L) at salinity 24 ppt. Following exposure, whole body copper accumulation and ions concentration (Na, K, Ca and Mg) were measured. Superoxide dismutase activity (SOD) and lipid peroxidation (LPO) were evaluated in muscle, liver and gills. Nuclear anomalies were also accessed in erythrocytes. Copper accumulation and whole body Mg concentration were dependent on copper concentration in the water ( $r^2=0.60$  and  $0.94$ , respectively). However, whole body Na, K and Ca concentrations, muscle LPO, SOD activity (muscle, liver and gills), and frequencies of erythrocytes with bilobed nucleus, binuclei and apoptotic fragments were not affected by copper exposure. In the other hand, a nonlinear relationship was observed between budding in erythrocytes and copper concentration. Also, a linear correlation between water copper concentration and micronuclei frequency ( $r^2=0.85$ ) in erythrocytes, liver LPO ( $r^2=0.80$ ) and gill LPO ( $r^2=0.68$ ) was observed. Taken altogether, results indicate that copper exposure is inducing physiological (whole body Mg concentration), biochemical (LPO) and genetic (micronuclei) damage in the tropical estuarine fish *P. vivipara*. These findings indicate that some biomarkers in different fish tissues are potentially suitable for monitoring ecologically relevant concentrations of copper in estuarine waters. Future studies will evaluate the reliability of the use of these biomarkers under field conditions. [Supported by the Brazilian CNPq (INCT-TA) and CAPES (Ciências do Mar)].

**TP181 Incorporating the 3R's While Improving Bioaccumulation Assessments for Product Stewardship** R. Hoke, DuPont, Haskell Global Centers; X. Han, DuPont, Haskell Global Centers, Haskell Global Centers; D. Nabb, R. Mingoia, DuPont, Haskell Global Centers. Product

stewardship is an integral, on-going aspect of environmental programs at major corporations. Animal welfare concerns as exemplified by the 3R's are an important consideration in product stewardship testing and the evolution of regulatory testing guidelines. Intelligent testing strategies that incorporate tiered testing and evaluation schemes help guide efficient use of limited resources, including test organisms, and facilitate scientifically-based chemical evaluations to ensure achievement of product stewardship and environmental protection goals. An example of a tiered assessment framework for bioaccumulation assessment is presented that incorporates data mining, modeling, physical-chemical property measurements, in vitro assays, and in-vivo toxicity tests to provide an assessment of bioaccumulation potential in fish. Examples of the application and limitations of the framework for bioaccumulation assessments will be presented including an evaluation of the animal welfare benefits of the framework and suggestions for additional research to improve the framework.

**TP182 Effects of Multi-walled Carbon Nanotubes on Germination, Growth, Water-use Efficiency and Photosynthetic Rate of Four Plant Species** B. Shrestha, Texas Tech Univ, The Institute of Environment and Human Health, Dept of Environmental Toxicology, Texas Tech Univ; P. Payton, USDA-ARS; J. Canas, Texas Tech Univ, Dept of Environmental Toxicol. Very few studies have been conducted on the effects of CNTs on plants. Existing studies on toxicity of CNTs on plants have shown mixed results of no effect, increased germination and growth, and inhibition in root elongation. However, all of these studies were conducted under different conditions on different plant species, which makes it difficult to compare the varying results. Thus, it is significant to do an extensive study on the effects of CNTs on different plant species. C4 and C3 plants differ in their photosynthesis pathways. In addition, C4 plants are able to grow in high temperature, high light conditions and have greater water-use efficiency. Since the past studies demonstrated variation in toxic effects of CNTs in different plant species, it is significant to compare the difference in CNTs toxicity on plant species that differ in their photosynthetic pathways. A series of experiments were conducted on the effects of multi-walled carbon nanotubes (MWCNTs) on germination, growth, water use efficiency and photosynthetic rate of different plants in soil. Two locally grown C3 plants (alfalfa and cotton) and two C4 plants (corn and sorghum) were chosen for this study. The study was conducted at MWCNTs soil concentrations of 0, 10, 100, 1000 and 10000 mg/kg. In a preliminary study conducted in pot, alfalfa and cotton percent germination decreased from the control to the highest concentration (mg/kg) by 27% and 80%, respectively. However germination increased in cotton by 55% from controls to a medium concentration (100 mg/kg). However, in corn and sorghum grown in petri dishes, germination occurred more rapidly and germination increased by 100% and 175%, respectively, from the control to the highest concentration (10000 mg/kg). No effects were observed during the preliminary study on water-use efficiency in alfalfa. A root inhibition and germination study will be conducted in an incubator at 24 °C for five days with all four plant species. Total germinated seedlings and shoot and root lengths will be quantified at the end of each study. Plants (alfalfa, corn and cotton) will be grown for three months to evaluate the effects on photosynthetic rate and long term growth. Shoot length, visual symptoms of toxicity and photosynthetic rate will be measured every week for eight weeks. This study will provide valuable information on the effects of MWCNTs on different plant species and allow for a comparison of the effects of MWCNTs on C3 and C4 plants.

**TP183 Flow Visualization Study of Interactions Between Nanoparticles and Air Bubbles** S. Jeong, Kunsan National Univ, Dept of Environmental Engineering; J. Hur, Kunsan National Univ; A.A. Keller, Univ of California-Santa Barbara. Nanoparticles released into the environment can accumulate on the soil layers or infiltrate into the groundwater. Nanoparticles traveling through the environment may be hazardous to humans and the environment. Although nanomaterial hazards have been much researched recently, the transport and fate of nanomaterials in the environment have not received sufficient attention. Fate and transport of nanoparticles flowing through soil and groundwater would be affected by the presence of unsaturated conditions or air bubbles in the flow paths. This study investigated interactions between nanoparticles and air by analyzing visualizations of nanoparticle aggregation and deposition on the air-water. The 2-dimensional heterogeneous type porous medium was manufactured by etching glass plates. Suspended nanoparticles of fullerene and zinc oxide were separately injected into the partially saturated porous medium and their transport was

investigated as time elapsed. Air bubbles significantly affected transport of nanoparticles through the flow paths. Nanoparticles were easily deposited on the interfacial thin flow paths between solid grains and air bubbles, which significantly affected their transport. However, the intermittent movement of air bubbles accelerated nanoparticle transport through preferential flow paths and resulted in breakthrough of the nanoparticles.

**TP184 Fundamental Assessment of Relationship Between Asian Dust and Influenza Infection-1: Immunotoxicity of Asian Dust for the Future Risk Analysis** Y. Yoshioka, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka Univ, The Center for Advanced Medical Engineering and Informatics; M. Fujimura, K. Yamashita, K. Higashisaka, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, National Institute of Biomedical Innovation, Laboratory of Biopharmaceutical Research; Y. Morishita, Osaka Univ, Laboratory of Toxicology and Safety Science, National Institute of Biomedical Innovation, Laboratory of Biopharmaceutical Research; P. Huiyan, T. Ogura, H. Nabeshi, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, National Institute of Biomedical Innovation, Laboratory of Biopharmaceutical Research; K. Nagano, Y. Abe, National Institute of Biomedical Innovation, Laboratory of Biopharmaceutical Research; H. Kamada, S. Tsunoda, National Institute of Biomedical Innovation, Laboratory of Biopharmaceutical Research, Osaka Univ, The Center for Advanced Medical Engineering and Informatics; M. Nasu, Osaka Univ, Laboratory of Environmental Science and Microbiology, Graduate School of Pharmaceutical Sciences; N. Itoh, T. Yoshikawa, Y. Tsutsumi, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, National Institute of Biomedical Innovation, Laboratory of Biopharmaceutical Research. Asian dust has been public concern about their potential risks to human health. Epidemiological studies have suggested that increased levels of Asian dust are associated with adverse effects in the respiratory and cardiovascular systems. However the immunological mechanism of Asian dust-induced exacerbations of allergic airway diseases or viral infection is not fully understood. Therefore, it is essential to examine the biological effects of Asian dust for the prevention of Asian dust-related diseases. Here, we examined the inflammatory effects of Asian dust in vitro, and we investigated the mechanisms by which Asian dust-induced inflammation. Asian dust collected in China and soil collected in dust source region (Loess Plateau, China) were used. At first, we compared the inflammatory effects of Asian dust and soil of Loess Plateau in vitro. We incubated mouse macrophage cells, RAW264.7 cells, with Asian dust or soil of Loess Plateau and measured tumor necrosis factor- $\alpha$  (TNF) production in the culture supernatant, because TNF is a crucial modulator of inflammation. Asian dust induced higher production of TNF than did soil of Loess Plateau. In addition, purified particle from soil of Loess Plateau with a diameter < 10  $\mu$ m induced higher production of TNF than did soil of Loess Plateau, indicating that small particles in Asian dust might possess a more potent inflammatory effect than did the larger particles in Asian dust. Next, to investigate the mechanisms of Asian dust-induced inflammation, we analyzed TNF production in RAW264.7 cells treated with Asian dust in the presence of an inhibitor of mitogen-activated protein kinases (MAPKs), such as ERK or JNK. Asian dust-induced TNF production was almost completely suppressed by the inhibitors, indicating that Asian dust-induced TNF production was mediated by ERK and JNK. Next, to investigate the involvement of reactive oxygen species (ROS) in Asian dust-induced TNF production, we measured the TNF production induced by Asian dust in the presence of ROS inhibitor. ROS inhibitor significantly suppressed Asian dust-induced TNF production, suggesting that Asian dust-induced production of ROS plays an important role in inflammatory responses. These results provide basic information for the prevention of inflammatory disease induced by Asian dust. Acknowledgement: This research was supported by the Environment Research and Technology Development Fund of the Ministry of the Environment, Japan.

**TP185 Fundamental Assessment of Relationship Between Asian Dust and Influenza Infection-2: Biodistribution of Asian Dust for the Future Risk Analysis** K. Yamashita, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences; Y. Yoshioka, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka Univ, The Center for Advanced Medical Engineering and Informatics (MEI center); M. Fujimura, Osaka Univ,

National Institute of Biomedical Innovation (NiBio); K. Higashisaka, Y. Morishita, H. Pan, T. Ogura, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR); H. Nabeshi, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences; K. Nagano, Y. Abe, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR); H. Kamada, Osaka Univ, The Center for Advanced Medical Engineering and Informatics (MEI center), National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR); S. Tsunoda, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR), Osaka Univ, The Center for Advanced Medical Engineering and Informatics (MEI center); M. Nasu, Osaka Univ, Laboratory of Environmental Science and Microbiology, Graduate School of Pharmaceutical Sciences; N. Itoh, T. Yoshikawa, Y. Tsutsumi, Osaka Univ, Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR). Summary: The health impact of Asian dust events has been public concern within China and in its neighboring countries. It is known that Asian dust is involved in many diseases such as allergic disease, cardiovascular disease or viral infection by epidemiological studies. However, little is known about detailed immunological mechanism of Asian dust-induced exacerbations of allergic airway diseases or viral infection. Therefore, it is essential to examine the biodistribution and biological effects of Asian dust for the prevention of Asian dust-related diseases. In this study, we examined the biodistribution and inflammatory effects of Asian dust in vivo. Asian dust collected in China and soil collected in dust source region (Loess Plateau, China) were used. At first, we examined the biodistribution of Asian dust and soil of Loess Plateau following intranasal injection by transmission electron microscope (TEM). BALB/c mice were treated with 30  $\mu$ g/mouse of Asian dust or soil of Loess Plateau via intranasal injection. After 24 h, tissues and organs, such as lung, spleen, liver, brain and nasal cavity, were analyzed by TEM. TEM analysis revealed that small particles of soil of Loess Plateau were found in surface of alveolar epithelial cells. Furthermore, small particles of Asian dust were found in surface and cytosol of the alveolar epithelial cells. No particles were seen in the spleen, liver, brain and nasal cavity of mice treated with Asian dust or soil of Loess Plateau. However, purified particle from soil of Loess Plateau with a diameter

**TP186 Influence of Multi-walled Carbon Nanotubes (MWNTs) on Sorption and Desorption of Polyaromatic Hydrocarbons (PAHs) in Soil** S. Li, Texas Tech Univ/The Institute of Environmental and Human Health, Environmental Toxicology; T. Anderson, Texas Tech Univ/The Institute of Environmental and Human Health, 1002 Frankford #1122B; J. Canas, Texas Tech Univ/The Institute of Environmental and Human Health. Carbon nanotube (CNT) containing products are available in US markets and have various applications, such as coatings, computers, clothing, wastewater treatment plants, medical devices and pesticides. An increase in CNT applications leads to the potential for CNTs to be released into the environment in large amounts. Therefore, studies related to CNT effects on ecosystems to verify their safety are necessary and vital. Previous studies have provided valuable information regarding the interactions between CNTs and organic contaminants, including PAHs. However, few published studies illustrate such interactions in a soil environment, which could provide more useful information about what might happen in a real soil environment. Recent studies estimated that nanotube concentrations in soil would be a factor of 20 higher than that in water. Thus, there is a need for such studies to be conducted in a terrestrial environment. Sorption tests were conducted by batch equilibrium method at room temperature. Briefly, PAHs in HPLC-grade methanol were added to a background solution containing 5 mM CaCl<sub>2</sub> and 1 $\times$ 10<sup>-5</sup> mM HgCl<sub>2</sub> to maintain a constant ionic strength and to inhibit microbial activity, respectively. Methanol concentrations were maintained at less than 0.1% (v/v) and pH was adjusted to pH 7.0 for all experiments. PAHs were added to 40 mL glass screw cap centrifuge tubes containing previously weighed samples. Tubes were agitated at 25 °C in the dark at 250 rpm for four days and then centrifuged for 20 minutes at 10000g. Supernatant was removed using a glass pipette and replaced with fresh background solution. Tubes were then further agitated under the same conditions described above. One sequential desorption step was performed. Preliminary data showed the ability of MWNTs to adsorb phenanthrene



dramatically decreased in a sandy loam soil. Additional studies with more PAHs and soil types are currently being conducted.

**TP187 Nanoparticle Toxicity and Bioavailability in *Hyaella azteca* Revealed by Ecotoxicogenomics** H.C. Poynton, US Environmental Protection Agency, Molecular Indicators Research, Univ of Massachusetts-Boston, Environmental, Earth and Ocean Sciences; J.M. Lazorchak, US Environmental Protection Agency, Office of Research and Development, USEPA Office of Research and Development, National Exposure Research Laboratory, USEPA, Molecular Ecology Research Branch; C. Impellitteri, US Environmental Protection Agency, Water Supply and Water Resources Division; M. Smith, The McConnell Group c/o USEPA ORD Cincinnati, Aquatic Toxicology Dept; H. Hering, The McConnell Group; C. Vulpe, Univ of California – Berkeley, Nutritional Science and Toxicology; J.K. Colbourne, Indiana Univ, and Mount Desert Island Biological Laboratory, The Center for Genomics and Bioinformatics; M.S. Sepulveda, Purdue Univ, Forestry & Natural Resources and School of Civil Engineering, Purdue Univ, Forestry & Natural Resources & Nanoparticles (NPs) are expected to make their way into the aquatic environment where sedimentation of particles will likely occur in most aquatic environments putting benthic organisms in these areas at particular risk. Therefore, organisms such as *Hyaella azteca*, an epibenthic amphipod which forages at the sediment surface, is likely to have a high potential exposure. Previous research has shown that *H. azteca* is several times more sensitive to zinc oxide (ZnO) NPs compared with the water column dweller, *Daphnia magna* perhaps due to the settling of the NPs. Once the particles settle on the sediment surface, sediment composition will likely play a role in the bioavailability and toxicity of the NPs. Understanding how water chemistry and sediment composition affects the bioavailability of NPs will help to define which organisms and ecosystems are most vulnerable to nanomaterials. Recently, we developed an oligonucleotide microarray for *H. azteca*, to better understand the mechanism of toxicity of NPs to sediment-dwelling organisms and identify biomarkers of exposure to nanomaterials. Transcriptomic analysis was performed to reveal genes which respond specifically to ZnO NP and Ag NP exposure and can be used to distinguish exposure between the nanoparticles and their metal ions. Further development of these biomarkers will be used to assess the bioavailability of NP to *H. azteca* in sediments of varying composition.

**TP188 Promotion of Antigen-specific IgE Production by Intranasally Administrated Nanosilica Particles in Mice** T. Yoshida, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR), Graduate School of Pharmaceutical Sciences, Osaka Univ, Laboratory of Toxicology and Safety Science; Y. Yoshioka, Graduate School of Pharmaceutical Sciences, Osaka Univ, Laboratory of Toxicology and Safety Science, Osaka Univ, The Center for Advanced Medical Engineering and Informatics (MEI\_center); M. Fujimura, S. Tochigi, T. Hirai, M. Uji, K. Misato, Graduate School of Pharmaceutical Sciences, Osaka Univ, Laboratory of Toxicology and Safety Science, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR); H. Takahashi, Graduate School of Pharmaceutical Sciences, Osaka Univ, Laboratory of Toxicology and Safety Science; A. Uda, K. Ichihashi, T. Mori, T. Akase, H. Nabeshi, Graduate School of Pharmaceutical Sciences, Osaka Univ, Laboratory of Toxicology and Safety Science, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR); K. Nagano, Y. Abe, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR); H. Kamada, S. Tsunoda, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR), Osaka Univ, The Center for Advanced Medical Engineering and Informatics (MEI\_center); N. Itoh, T. Yoshikawa, Y. Tsutsumi, Graduate School of Pharmaceutical Sciences, Osaka Univ, Laboratory of Toxicology and Safety Science, National Institute of Biomedical Innovation (NiBio), Laboratory of Biopharmaceutical Research (LBR). With the recent development of nanotechnology, many nanomaterials (NMs) with innovative functions have been developed. For example, nanoparticles of titanium dioxide and silica have been used in commercial applications related to medicine, cosmetics and food. In particular, amorphous nanosilica particles (nSPs) possess extraordinary advantages, including straightforward synthesis, relatively low cost, and easy surface modification. nSPs are increasingly being used for many applications, including cosmetics, food technology, medical diagnosis. As the use of NMs increases, there is rising concern regarding their potential health risks because there is preliminary evidence that the unique electrical and mechanical properties

of NMs is associated with undesirable biological interactions. However, few studies have assessed the role of the different physical characteristics of NMs, including nSPs, in allergic responses. Here, we examined whether intranasally-administered various sizes of silica particles have the capacity to promote allergic immune responses in mice. We used nSPs with diameters of 30 or 70 nm (nSP30 or nSP70, respectively), and conventional micro-sized silica particles with diameters of 300 or 1000 nm (nSP300 or mSP1000, respectively). Mice were intranasally exposed to chicken egg ovalbumin (OVA) plus various sizes of silica particles, and the levels of OVA-specific antibodies (Abs) in the plasma and the release profiles of cytokines from the splenocytes were determined. Intranasal exposure to OVA plus smaller nSP tended to induce a higher level of OVA-specific immunoglobulin (Ig) E, IgG and IgG1 Abs than did exposure to OVA plus larger silica particles. Splenocytes from mice exposed to OVA plus nSP30 secreted higher levels of Th2-type cytokines (IL-4, IL-5) than mice exposed to OVA alone. Taken together, these results indicate that nSPs below 100 nm can induce allergen-specific Th2-type immune responses in vivo. We believe that these data should provide basic information that can help risk analysis of NMs. Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan. Reference: Yoshida Y., Yoshioka Y., et al: Promotion of allergic immune responses by intranasally-administrated nanosilica particles in mice. *Nanoscale.Res.Lett.*, in press.

**TP189 The Effects of Multi-walled Carbon Nanotubes on Soil Microbial Communities** B. Shrestha, Texas Tech Univ, The Institute of Environment and Human Health, Dept of Environmental Toxicology, Texas Tech Univ; V. Acosta-Martinez, USDA-ARS; S. Cox, Texas Tech Univ, Dept of Environmental Toxicology; J. Canas, Texas Tech Univ. Applications of nanomaterials, including carbon nanotubes (CNTs), are increasing; however, their impact on the environment is still not well understood. Microbial toxicity of CNTs in pure culture conditions has been reported, however, it is crucial to assess their risks beyond pure culture in an actual ecosystem. A semi-arid agricultural sandy soil was treated with multi-walled carbon nanotubes (MWCNTs) at four different concentrations (10-10000 mgMWCNTs kg<sup>-1</sup>soil), and incubated with a control (soil only) in the dark at 23°C for 90 d. Soil microbial-basal respiration, enzymatic activities and microbial community structure were evaluated. There was no significant difference in soil basal respiration monitored every week for 28 d among the different treatment groups and control. Acid phosphatase activity was affected after 28 d, but no significant effects were observed on  $\beta$ -glucosidase and  $\beta$ -glucosaminidase activities. There was an overall decrease in all enzyme activities after 90 d; however,  $\beta$ -glucosidase and  $\beta$ -glucosaminidase activities were higher in the 10000 mgMWCNTs kg<sup>-1</sup>soil treatment than the control and other treatments. Also, the effect of MWCNT concentration on acid phosphatase activity observed on day 28 subsided after day 90. Dehydrogenase activity was very low in the 10000 mgMWCNTs kg<sup>-1</sup>soil treatment on days 28 and 90; however activity was lower in the controls than the 10- and 100 mgMWCNTs kg<sup>-1</sup>soil treatments on day 90. Separate tests demonstrated an interference of the MWCNTs ( $\geq 10000$  mgkg<sup>-1</sup>soil) with the measurement of dehydrogenase activity by affecting absorbance of a color product. Total microbial biomass, determined with the sum of fatty acids methyl esters (FAMES), was highest in the 10000 mgMWCNTs kg<sup>-1</sup>soil treatment for both 28 and 90 d samples. Principal component analysis of FAME profiles for both 28 and 90 d samples showed separation of 10000 mgMWCNTs kg<sup>-1</sup>soil treatment from the control along principle component 1 (89% and 80% of variance respectively). This study suggests the presence of MWCNTs may cause shifts in soil microbial community structure and enzyme activities (Carbon and Nitrogen cycling). However, an extensive study is required with evaluation of other components of soil ecosystems to support the preliminary results. The study will be able to provide valuable information on the effects of carbon nanotubes on soil microbial communities, which will be valuable in risk assessments of carbon nanotubes.

**TP190 Trophic Transfer of Au Nanoparticles Along a Simulated Terrestrial Food Chain** J.M. Unrine, Univ of Kentucky, Dept of Plant and Soil Sciences, Univ of Kentucky, Dept of Plant & Soil Sciences; W.A. Shoultz-Wilson, Roosevelt Univ, Dept of Biological, Chemical and Physical Sciences; O. Tsyusko, Univ of Kentucky, Dept of Plant & Soil Sciences. Recent studies have suggested that terrestrial environments will serve as the ultimate sink for several commonly used engineered nanoparticles (NPs),



primarily through application of sewage sludge applied as biosolids to agricultural soils. Little is known concerning the fate and adverse ecological effects of nanomaterials in terrestrial ecosystems. Our previous studies demonstrate that detritivores (earthworms) accumulate manufactured nanoparticles from soil in their tissues. In this study, we used Au NPs as a stable probe to determine if nanoparticles in soil can be taken up by detritivores (*Eisenia fetida*) and transmitted to carnivores (*Rana catesbeiana*) in a simulated laboratory food chain. Au NPs were used in order to circumvent difficulties involved in differentiating uptake of intact particles from dissolution products since they are redox stable under ambient conditions. We also tested the hypothesis that Au NPs are more bioaccumulative through trophic exposure than direct exposure by exposing bullfrogs to Au NPs either through the simulated food chain (soil-earthworm-bullfrog) or directly via oral gavage. Gold concentrations in soil were approximately 200 mg/kg. Accumulation of Au NPs in earthworms was characterized by a rapid increase in whole body concentrations for the first 10 days, followed by a rapid decrease in body concentrations over the subsequent 10 days. Whole body concentrations ranged from < 1 ug/g fresh mass on day one to 9.5 ug/g FM at the peak of uptake on day 10. Following transfer to clean soil media, body concentrations did not decrease over a 60 day period indicating slow or nonexistent elimination. Gold was detected in all of the organs of the exposed bullfrogs. The highest concentrations were found in spleen, and they exceeded 2 ug/g dry mass in some samples. The remaining organs had concentrations decreasing in the following order (intestine>kidney>stomach>liver>muscle) and ranged from 24.8 ng/g to 2.4 ug/g. The results demonstrate that, trophically exposed bullfrogs accumulated twice as much of the applied dose than the directly exposed bullfrogs. This suggests that nanoparticles are transformed in soil and/or along the food chain in a way that enhances their bioavailability. We did not observe evidence for biomagnification as in our previous study with tobacco horn worms.

**TP191 Assessment of Immunological Health and Mercury Exposure in Common Loons of the Adirondack Mountains** K. Grasman, Calvin College, Dept of Biology, Calvin College, Dept of Biological; J. Singer, R. Abma, Calvin College, Dept of Biology; N. Schoch, Biodiversity Research Institute. Exposure of common loons (*Gavia immer*) to mercury through atmospheric deposition and biomagnification is a concern throughout the upper Midwestern and Northeastern US and Canada. This study assessed potential associations between mercury exposure and immune function in wild loons in the Adirondack Park of upstate New York using lymphocyte cryopreservation and in vitro proliferation assays. During 2008-09, loons were spotlighted and netted at night for collection of heparinized blood. During 2008 lymphocytes were isolated within six hours of collection using a slow spin technique at several centrifuge speeds to optimize lymphocyte collection. White blood cells were counted in the field using a hemacytometer using Natt-Herrick's stain. The slow spin method (150xg) worked well in loons, although thrombocyte and heterophil contamination was higher than in other species. In both years lymphocyte samples were frozen in culture medium with 10% DMSO and stored in liquid nitrogen. Samples were thawed, and proliferation lymphocytes was induced via mitogens (phytohemagglutinin (PHA), Concanavalin-A (Con-A), lipopolysaccharide (LPS), and phorbol myristate acetate (PMA)). Proliferation was assessed by an ELISA measuring the incorporation of bromodeoxyuridine (BrdU) into newly synthesized DNA. Storage of lymphocytes for up to 22 months did not significantly reduce post-thaw viability. Proliferation assays demonstrated low replicate variability and high reproducibility. In adult loons lymphocyte proliferation indices were not directly associated with blood mercury, nor any other contaminants (selenium, DDE and PCBs). The proliferation of T lymphocytes in response to PHA + PMA was elevated nonsignificantly in low pH lakes (< 6.3) and significantly during 2009. Lakes sampled in 2009 had lower pHs than in 2008. In chicks, blood mercury showed nearly linear relationships with several lymphocyte proliferation indices, but evaluation of these potential relationships was hampered by low sample sizes. Hence, future investigations should emphasize greater sampling of loon chicks. Potential relationships between altered immune function and mercury exposure in young loons are consistent with the general sensitivity of the developing immune system to contaminants and with a previous laboratory study that demonstrated immunotoxicity of environmentally relevant exposures of mercury in young loons.

**TP193 Effects of Environmentally Relevant Levels of Methylmercury on Flight Performance in European Starlings (*Sturnus vulgaris*)** J.R.

Carlson, D. Cristol, J. Swaddle, The College of William and Mary, Biology. Recent studies indicate that methylmercury bioaccumulates and biomagnifies in terrestrial habitats. Unusually high blood mercury has been reported in multiple passerine species living in contaminated areas, and in some cases these levels equaled or exceeded levels of piscivores in the same area. Despite these findings, little is known about the biological consequences of sub-lethal, ecologically relevant mercury levels in songbirds. Flight performance was analyzed in European Starlings (*Sturnus vulgaris*) dosed with environmentally realistic levels of methylmercury-cysteine. Sixty birds were treated with dietary doses of 0.00ppm, 1.5ppm or 3.0ppm, but after four weeks on treatment three birds in the high dosage group died of kidney failure—an interesting result in itself. After two weeks off treatment, the levels were then adjusted to 0.00ppm, 0.75ppm, and 1.5ppm. Birds were tested pre-dosage and after their blood mercury levels plateaued for (1) Take-off angle and speed combined into a metric of energy gained during flight in response to a predator stimulus and (2) Velocity and angle while maneuvering around an obstacle in the flight path. Continuous retesting will establish longer term effects of chronic exposure on flight performance. Environmentally relevant levels of dietary methylmercury are predicted to cause decreased flight performance in European Starlings due to potential disruption of neuromuscular functioning, bioenergetics, and behavior that have been observed in multiple species at similar or higher doses. Flight is of paramount importance for fitness because it plays a critical role in virtually all life history traits of a typical songbird.

**TP194 Effects of Mercury on Yellow Perch Reproductive and General Health in Kejimikujik National Park and Historic Site, Nova Scotia** K. Fischer, Canadian Rivers Institute, Univ of New Brunswick; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology; K. Munkittrick, Canadian Rivers Institute, Univ of New Brunswick; N. Burgess, Environment Canada, Ecotoxicology & Wildlife Health Division; P. Drevnick, Université du Québec, INRS-ETE. Kejimikujik National Park and National Historic Site (KNPNHS), located in southwestern Nova Scotia, Canada, has been designated a biological Hg "hotspot" due to the high concentrations of this pollutant in common loons (*Gavia immer*) and their main prey yellow perch (*Perca flavescens*). In KNPNS concentrations of total Hg in yellow perch have increased over the last decade, and many currently exceed the tissue threshold level estimated for adverse effects in juvenile and adult fish (0.2 µg Hg g<sup>-1</sup> (ww)). Our objective was to determine whether elevated Hg is adversely affecting the general and reproductive health of yellow perch. To determine this we collected male and female perch in the fall of 2009 and 2010 from 12 lakes known to contain fish with differing Hg concentrations. Several health variables (condition, liver somatic index (LSI), gonadosomatic index (GSI), macrophage aggregates, oocyte development, sex steroid concentrations and gene expression in the liver) were examined in these fish, compared between lakes, and related to tissue Hg concentrations. Across lakes, mean total mercury concentrations ranged from 0.29 – 0.55 µg Hg g<sup>-1</sup> (ww) (n = 14 – 46 fish/lake) in dorsal muscle and from 0.26 – 2.12 µg Hg g<sup>-1</sup> (ww) (n = 11 – 23 fish/lake) in liver of the perch. Additionally, in the liver the mean % total Hg present as methyl Hg ranged from 28.3-66.5% across lakes (n=11 – 23 fish/lake). Significant among-lake, within-sex differences in condition and GSI do not appear to be related to tissue Hg concentrations, and no effects of Hg on oocyte development were found. However, LSI was negatively related across lakes to total Hg in livers of perch ≤ 1 year old, and the proportion of macrophage aggregates (an indicator of oxidative stress and tissue damage) found in the liver, kidney and spleen were positively related to tissue total Hg for all ages. Analyses of plasma sex steroids, and the expression of stress related genes in the liver, are ongoing. We concluded that elevated Hg concentrations in KNPNS yellow perch are associated with adverse general health effects at the cellular and organ level. The lower range of Hg concentrations found in KNPNS perch is common among wild yellow perch populations of Northeastern North America. Given that Hg is global pollutant, this raises concern for the health of this species as well as other wild fishes.

**TP195 Mercury and Selenium Bioconcentration, Bioaccumulation, and Biomagnification in Sarasota Bay Ecosystems** Y. Hong, Johns Hopkins Univ, Geography and Environmental Engineering; E. Rifkin, National Aquarium Baltimore, National Aquarium Conservation Center; R. Wells, Chicago Zoological Society, Mote Marine Laboratory; P. Hull, Mote Marine Laboratory; E. Bouwer, Johns Hopkins Univ, Geography and Environmental Engineering. Mercury (Hg) is a highly toxic metal and readily

accumulates in biota, hence elevated Hg concentrations are often observed in fish raising a significant health concern for piscivorous animals. To better understand Hg distribution and to better assess the risk associated with dietary mercury exposure in ecosystems, a field experiment was conducted in Sarasota Bay, FL. Published studies have suggested that selenium (Se) in diets can antagonistically reduce the toxicity of mercury, so Se distribution was also investigated. Diffusive gradient in thin film probes were deployed to determine labile methylmercury (MeHg) and total mercury (THg) concentrations in the overlying water and in sediment porewater in situ. Environmental samples, including water, sediment, clams (*Pinna nobilis*, *Mercenaria mercenaria*, *Venus affinis*), plankton, and seagrass (*Thalassia testudinum*), were collected from several locations in the area and analyzed for THg, MeHg, and Se. THg and Se levels in fish and Sarasota Bay resident bottlenose dolphins were obtained from previously published work. The trophic levels of the collected samples were characterized by nitrogen isotope ( $\delta^{15}\text{N}$ ) analysis. From the results, bioconcentration factors of THg, MeHg, and Se from water to plankton, sediment to benthic organisms, and sediment porewater to benthic organisms, were evaluated and compared with published values reported from other environments. To compare biomagnification across food webs, the linear relationships between Log (THg and MeHg) and  $\delta^{15}\text{N}$  were evaluated and the regression slopes and the intercepts were used as a measure of biomagnification rate and the baseline values for primary producers, respectively. The results showed between a 4 and 6 orders-of-magnitude increase in the Se and THg concentrations from the environmental matrix, such as water and sediment, to the Sarasota resident bottlenose dolphins' blood and skin tissue samples. The results suggested that the co-accumulation and biomagnification of Hg and Se is occurring in the food web of Sarasota Bay and that the top predators in aquatic ecosystems may encounter potential health risks associated with dietary Hg exposure even in a relatively uncontaminated coastal area.

**TP196 Mercury and Selenium in Sharks of the Northeastern United States: A Multi-species Approach Towards Potential Sub-lethal Effects** D.H. Adams, Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, Cape Canaveral Scientific, Inc., Fish & Wildlife Research Institute; D. Nam, Univ of Michigan, Dept of Environmental Health Sciences; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. There continues to be concerns about mercury in the US Atlantic ecosystem, with regard to health of marine biota, as well as human consumers. Dorsal muscle, brain, and liver were collected from a wide array of shark species from Atlantic Ocean offshore waters of the northeastern United States, including shortfin mako shark (*Isurus oxyrinchus*), porbeagle (*Lamna nasus*), common thresher shark (*Alopias vulpinus*), blue shark (*Prionace glauca*), smooth dogfish (*Mustelus canis*), blacktip shark (*Carcharhinus limbatus*), sandbar shark (*Carcharhinus plumbeus*), dusky shark (*Carcharhinus obscurus*), scalloped hammerhead (*Sphyrna lewini*), and tiger shark (*Galeocerdo cuvier*). These mercury results, coupled with data regarding diet, feeding ecology, and life history parameters of these species, provide further insight into mercury bioaccumulation in this large marine region. We found strong positive correlations between mercury and shark size, and clear linkages between mercury concentrations and trophic position of these species within the US Atlantic ecosystem. Total mercury in several of these important apex predators can exceed current US and international regulatory guidelines. Preliminary results for shortfin mako sharks suggest there are sub-lethal neurochemical effects directly related to mercury concentrations. We will also report selenium concentrations for a representative group of these shark species and provide insight into mercury-selenium interactions in these important apex predators.

**TP197 Mercury Contamination in Turtles of Eastern Oklahoma: Food Consumption Advisories and Nonlethal Tissue Analysis** A.L. Powell, Oklahoma State Univ, Dept of Zoology; J.R. Bidwell, School of Environmental and Life Sciences; J.B. Belden, Oklahoma State Univ, Dept of Zoology. Mercury is a naturally occurring element, however its persistence as an anthropogenic pollutant continues to adversely affect wildlife. Significant research effort has focused on characterizing mercury residues in fish tissue due to the high consumption of fish by humans. For example, according to the Oklahoma Dept of Environmental Quality, the results of 3 years of testing predator fish in the lakes of Oklahoma indicate at least 16 lakes have fish mercury levels exceeding safe consumption levels. Much less work has been conducted on turtles despite the predominately aquatic

nature of many species, their relatively high trophic status, and the fact that turtles are also harvested for human consumption in some parts of the United States. Furthermore, studies designed to measure mercury in turtles are often complicated by necessity to sacrifice individuals from long-lived and slowly reproducing species. Thus, the objectives of this study were to 1) determine if mercury concentrations in two freshwater turtle species of Oklahoma, *Trachemys scripta* and *Chelydra serpentina*, are high enough to necessitate consumption advisories for turtles; 2) determine if concentrations in tissues that can be collected through nonlethal means (scute, tail clip, claw) adequately predict concentrations in tissues that likely require lethal sampling (muscle and liver). Regardless of the tissue used, mercury analysis was accomplished by traditional wet digestion followed by CVAAS. Turtles were collected across 8 sites (rivers and lakes) all located in Eastern Oklahoma. Hg levels in leg muscle differed among sites ( $P=0.0167$ ) but not between species, sex, or size. Hg levels in liver tissue also differed among sites ( $P=0.0172$ ), but were not equivalent to site differences in leg muscle. Based on the EPA's food consumption guidelines, we calculated elevated Hg concentrations in turtle leg muscle samples, eliciting consumption limits ( $>0.1 \text{ ug/kg day}^{-1}$ ) at 2 of the 6 sites analyzed thus far. Claw and muscle Hg concentrations were strongly correlated ( $R^2=0.81$ ,  $P<0.0001$ ), suggesting that claw is a useful indicator of muscle Hg burden, which can be sampled using non-lethal sampling techniques.

**TP199 The Deposition of Mercury Through Throughfall and Litterfall in Yangsu-ri Forest, Korea** M. Kim, J. Han, S. Jung, S. Yi, Seoul National Univ; K. Zoh, Seoul National Univ, Seoul National Univ, Dept of Environmental Health, School of Public Health. Mercury deposition to the landscape is typically evaluated using data from precipitation Hg deposition. Litterfall is an important flux of mercury (Hg) to soils in forested landscapes, yet much less is known about litterfall Hg. We measured dry and wet deposition, throughfall, litterfall, soil, and soil water of Hg contributions in Yangsu-ri forest, Gyeonggi-Do, Korea, in order to investigate how the Hg species present in the atmosphere is being transferred to other media. Dry and wet deposition and throughfall samples have been collected every two weeks from September 2008 to February 2010 and analyzed for total mercury (THg). Litterfall and soil have been sampled monthly from December 2008 to October 2010 and analyzed for THg. Soil water has been sampled using replicate zero tension lysimeters placed beneath the A horizon at four replicate plots. Soil water has been sampled monthly beginning in July 2009 and analyzed for Hg content. The reactive gaseous mercury (RGM) flux of dry deposition was  $1.16 \pm 0.34 \text{ ng m}^{-2} \text{ h}^{-1}$  in spring. The particulate mercury (Hgp) flux of dry deposition was  $1.11 \pm 1.05 \text{ ng m}^{-2} \text{ h}^{-1}$  in summer. The fluxed of precipitation and throughfall were 4.33 and  $6.41 \text{ } \mu\text{g m}^{-2} \text{ yr}^{-1}$ , respectively. Precipitation and throughfall were higher in the summer by rain. The THg in soil was the highest concentration in A horizon of fir habitat ( $77.82 \pm 3.04 \text{ } \mu\text{g kg}^{-1}$ ). Total litterfall fluxes were  $9.95 \text{ } \mu\text{g m}^{-2} \text{ yr}^{-1}$  for coniferous tree and  $4.61 \text{ } \mu\text{g m}^{-2} \text{ yr}^{-1}$  for deciduous tree. During the entire sampling period, the THg concentration of soil water was  $6.45 \pm 6.25 \text{ ng L}^{-1}$ . Based on the measured result in this study, rate of wet deposition, throughfall, and litterfall was 1 : 1.48 : 2.30. The ratio was somewhat lower than those values reported in other studies, suggesting that this study site is clean, uncontaminated by pollutants, and has relatively little airborne mercury inputs.

**TP200 Where's the Mercury? Constructing and Applying Spatial Models to Identify Lakes with Potentially High Fish Hg Concentrations in New York, USA** M. Turnquist, State Univ of New York – College of Environmental Science and Forestry, Environmental Forest Biology, Biodiversity Research Institute, SUNY-ESF, Environmental Forest Biology; M.A. Schlaepfer, State Univ of New York – College of Environmental Science and Forestry, Environmental and Forest Biology; C. Driscoll, Syracuse Univ, Dept of Civil & Environmental Engineering. Numerous studies have examined the relationship of fish mercury (Hg) concentrations to environmental factors and here we increased model predictability by using landscape features depicted through GIS and atmospheric Hg deposition in New York (NY), USA. The objective was to construct models that integrate water chemistry and landscape features to empirically predict Hg in four standard-size fish species; yellow perch (229mm, *Perca flavescens*), smallmouth bass (356mm, *Micropterus dolomieu*), largemouth bass (356mm, *M. salmoides*), and walleye (457mm, *Sander vitreus*), and apply the models to NY lakes. Linear regression models were parameterized using 171 lakes with both fish Hg and environmental parameters. Akaike's information criterion and the

relative weight were used to determine the most suitable models. The prominent model parameters were the mean gaseous elemental Hg deposition, the % wet area relative to the lake, and acid neutralizing capacity, resulting in coefficients of determination ( $R^2$ ) as follows, yellow perch  $R^2 = 0.59$ , smallmouth bass  $R^2 = 0.64$ , largemouth bass  $R^2 = 0.39$ , and walleye  $R^2 = 0.15$ . The models were validated. In 359 NY lakes, fish Hg concentrations were predicted. Consumption risk levels were determined using the 95% prediction interval and where the mean predicted Hg concentration fell in relation to the USEPA Action Limit ( $0.3 \mu\text{g/g}$ ) for fish consumption advisories. For yellow perch, smallmouth bass, largemouth bass, and walleye, 34%, 80%, 87% and 97% of the 359 lakes, respectively, had mean predicted Hg concentrations above the USEPA Action Limit. Model outputs identified individual lakes as well as regions where fish Hg was likely to exceed safe consumption limits.

**TP201 Capabilities and Method Improvements of Single Particle (SP)-ICP-MS for the Detection of Silver Nanoparticles** D. Mitrano, Colorado School of Mines, Chemistry and Geochemistry; H. Pace, Colorado School of Mines, grad student; A. Barber, Colorado School of Mines, Dept of Chemistry and Geochemistry; C. Higgins, Colorado School of Mines, Environmental Science and Engineering; A.J. Bednar, US Army Engineer Research and Development Center; J. Ravnille, Colorado School of Mines, Chemistry and Geochemistry, Colorado School of Mines. One of the most rapidly growing classes of nanoproducts are consumer materials that generate silver ions or contain nanosilver. The environmental prevalence of nanomaterials, particularly nanosilver, is expected to increase substantially in the future. Thus, it is imperative to develop techniques capable of determining key characteristics of nanosilver in complex media including, but not limited to, wastewater and biological samples. Of the standard analytical techniques that currently exist, few seem fully capable of determining the concentration, size, and composition of this new class of emerging contaminant. Single Particle ICP-MS (SP-ICP-MS), a novel application of ICP-MS, provides number, size, and composition data for nanoparticles as small as 40nm at environmentally relevant concentrations as low as 25 ppt. Furthermore, the distinction and quantification of dissolved vs. nanoparticulate concentrations is advantageous over other techniques available; as each may behave differently in biological and environmental systems and this ratio may prove important in many instances. Specific method improvements will be discussed such defining the dissolved metal background, dwell time choice, and ICP-MS optimization for the SP-ICP-MS technique. Suggested developments were validated using well-characterized and stable silver nanoparticles of discrete sizes from 20 to 100nm and cross-checked on several ICP-MS instruments to determine sensitivity and applicability in various laboratories. The validation and application of this analytical technique will result in a powerful tool for the continued investigation of inorganic nanoparticulate behavior in both environmental and biological systems.

**TP202 Use of AF4-UV-Vis-MALLS/DLS-ICP-MS for Characterization of Silver Nanoparticle Behavior in Aquatic Microcosms** J.M. Unrine, Univ of Kentucky, Dept of Plant and Soil Sciences, Univ of Kentucky, Dept of Plant & Soil Sciences; B. Colman, A. Bone, C. Matson, Duke Univ, Center for Environmental Implications of Nanotechnology. Recent studies of silver nanoparticle (Ag NP) toxicity in aquatic mesocosms have suggested that toxicity is diminished relative to laboratory assays in simple media. In support of toxicity studies that aimed to identify factors contributing to this amelioration of toxicity, we investigated the aggregation and dissolution of silver nanoparticles in aquatic microcosms that contained components of the mesocosms. The aquatic microcosms contained either just mesocosm water (W), mesocosm water and sediment (WS), mesocosm water and aquatic plants (WP; *Egeria densa* and *Potamogeton diversifolius*) or water, sediment and plants (WSP). We analyzed particle size distribution using asymmetrical flow field flow fractionation (AF4) with UV-Vis diode array, multi-angle/dynamic light scattering (MALLS/DLS), and inductively coupled plasma mass spectrometry (ICP-MS) as detection methods. Microcosms contained either 2 mg/L of 60 nm polyvinylpyrrolidone (PVP) coated Ag nanoparticles, 6 nm gum Arabic (GA) coated Ag nanoparticles or Ag<sup>+</sup> from AgNO<sub>3</sub>. For the PVP Ag NPs, heteroaggregation was observed in the W and WS treatments, but not the WP or WSP treatments. Greater dissolution also occurred in the W and WS treatments. Few large Ag containing aggregates were detected in the GA treated waters from any treatment; however, concentrations of primary particles in the WP and WSP treatments were only about half of those in the W and WS treatments. The combination of sediment with

plants enhanced the reduction in suspended primary particle concentrations. We also observed binding of Ag<sup>+</sup> ions to dissolved organic matter (DOM) in the treatments containing plants. Microcosms containing Ag<sup>+</sup> and plants also displayed peaks for Ag bound to DOM. Overall, we observed that the presence of plants had a stabilizing effect on primary PVP Ag NP and a destabilizing effect on primary GA Ag NP concentrations in the water column. DOM from plants also bound considerable amounts of Ag<sup>+</sup> ions. These results may help to explain reductions in Ag NP toxicity observed in both microcosm and mesocosm studies relative to simple laboratory studies.

**TP203 Size and Composition Separation of Engineered Nanomaterials by Ultracentrifugation** J. Sylva, Student Services Contract to EPA; C.G. Rosal, G. Momplaisir, USEPA, ORD/NERL/ESD. The production of engineered nanomaterials (ENMs) has tremendously increased to satisfy their demand in technological, medical, pharmaceutical, and personal care products. This in turn translates into higher potential for these materials' release in the environment. Nanoparticles have unique chemical and physical characteristics depending on their sizes and shapes relative to their corresponding bulk materials. Determination of these intrinsic properties can be complicated due to the wide distributions of sizes. To determine potential exposure of metal- and metal oxide-based ENMs in aqueous matrices, we propose to use ultracentrifugation for size separation followed by elemental detection using inductively coupled plasma-mass spectrometry (ICPMS). Scanning electron microscope (SEM) and capillary electrophoresis (CE)-ICPMS will also be employed as confirmation and complimentary techniques. Preliminary results will be presented in this poster.

**TP204 Optimization of Pre-filtration of Surface Water for Quantification and Size Characterization of Engineered Nanoparticles** E.M. Siska, Student Contractor to the USEPA, USEPA; E.M. Heithmar, US Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, USEPA, Research Chemist. As quantities of metal and metal oxide based engineered nanomaterials (ENMs) in consumer products increase, higher loadings of released nanoparticles in wastewater and ultimately surface water will follow. Understanding their potential risk to human health and ecosystems requires information on both potential exposure and effects. To evaluate potential exposure, the concentration and size distribution of the nanoparticles in surface water is a critical piece of information. All existing analytical methods for quantifying and characterizing metal-bearing ENM particles require pre-filtration of most surface waters. Unfortunately, the nominal size cut-off of filtration media is a poor predictor of the recovery of nanoparticles from surface water. Previous results in this laboratory indicated good recovery of 50 nanometer silver and gold nanoparticles from reagent water, using a filter of 5  $\mu\text{m}$  cut-off. However, recovery of the same nanoparticles from urban stream water resulted in < 10% recovery. Filtration of nanoparticles from surface water can also potentially alter the size distribution. Here we report using single particle-inductively coupled plasma mass spectrometry to study the factors influencing recovery and size-distribution artifacts produced by filtration of surface water. The effects of nanoparticle composition, filtration media type, size cut-off, sample volume to filter surface area ratio, and other factors are investigated. Experimental conditions and expected data-quality for optimized pre-filtration of metal-bearing ENMs will be presented.

**TP205 Measuring the Bioconcentration of DOSS in Pacific Oysters** H. Boorse, Oregon Episcopal School, Science, Oregon Episcopal School, Columbia Analytical Services. The oil dispersants Corexit 9527<sup>®</sup> and 9500 A<sup>®</sup> were used in mass quantities in treatment of the Deep Water Horizon Gulf of Mexico oil spill of 2010. (Boxall, 2011) There has been much concern and controversy over the effect and toxicity of these oil dispersants to marine biota. Studies were conducted by EPA in 2010 on the toxicity of various oil dispersants and their oil mixtures. Primary active ingredients in oil dispersants are surfactants. To date, very little research has been conducted on the affect, toxicity or bioaccumulation of these surfactants on marine organisms. This investigation focused on the short term bioconcentration of Dioctyl Sulfosuccinate Sodium Salt (DOSS), the primary surfactant found in Corexit<sup>®</sup> products, in the Pacific oyster *Crassostrea Gigas*. DOSS is water soluble and fat soluble and has the potential to accumulate in the fatty tissues of organisms. Oysters were exposed to low-level concentration ( $\mu\text{g/L}$ ) of Corexit 9500 A<sup>®</sup> over an approximate 60 hour period of time. Oyster and water samples were taken at approximately six hour intervals, frozen and shipped to the laboratory for DOSS analysis by liquid chromatography



tandem mass spectroscopy (LC/MS/MS). The results of this biconcentration study are discussed.

**TP206 A Comparison of Patterns of PCB Congeners and Aroclor Mixtures in Sediments and Fish Tissues in the River Raisin, Monroe, MI, USA** S. Roark, CH2M Hill, Ecotoxicology & Risk Assessment; M. Loomis, USEPA, Great Lakes National Program Office. Bioaccumulation of polychlorinated biphenyls (PCBs) varies among congeners, with congeners of intermediate chlorination (4 to 7 chlorines) generally accumulating in fish tissue more than those of greater and lesser chlorination. Consequently, the profile of PCBs in fish tissue typically differs from that of sediments from the same location. Furthermore, reported congener profiles in fish tissue are only an approximate match for theoretical congener profiles based on proportional technical mixtures of Aroclors. Data available from the USEPA Great Lakes National Program Office for River Raisin (RR) indicated that Aroclor 1242 is the predominant Aroclor mixture in RR sediment, with Aroclors 1254 and 1260 infrequently detected or representing only a small percentage of the total PCB. Consistent with the technical mixture of Aroclor 1242, congeners with 3 or fewer chlorines composed approximately 60 percent of total PCB, and congeners with 4 or fewer chlorines composed approximately 90 percent of the total PCB in RR sediment data reported from 2001. Despite consistent profiles in RR sediments, reported PCB Aroclor profiles in fish tissue were variable, and congeners with 4 or fewer chlorines represented a lesser proportion of the total PCB than was reported in sediments. Fillet data reported from the Michigan Dept of Environmental Quality Fish Contaminant Monitoring Program indicated (FCMP) that in 1998 smallmouth bass, freshwater drum, and carp tissues from RR contained only Aroclor mixtures 1248 and 1254. PCB congener analyses from the FCMP for several fish species analyzed in 2008 indicated that PCB congeners with 4 or fewer chlorines represented 23 to 40 percent or less of the total PCB in RR fish tissues, although only 73 congeners (or coelution groups) were reported. Additional contemporaneously collected sediment and fish tissue data, including Aroclors and congeners, will further elucidate these patterns. An understanding of the differential bioaccumulation of PCB congeners may be important in ecological risk assessments for fish and consumers of fish, because congeners of intermediate chlorination include those of greatest toxicological significance and are accumulated disproportionately in fish tissues.

**TP207 Accumulation and Depuration of Polychlorinated Biphenyls from Field-collected Sediment in Three Freshwater Organisms** L. Van Geest, Univ of Guelph, School of Environmental Sciences, Intrinsic Environmental Sciences Inc; D. Poirier, Ontario Ministry of the Environment, Laboratory Services Branch; P. Sibley, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Dept of Environmental Biology; K. Solomon, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Centre for Toxicology, School of Environmental Sciences. As laboratory-based bioaccumulation methods are standardized and expanded to include other test species, kinetic studies assessing the major classes of contaminants with these species are needed to support and/or improve the selection of standard duration for bioaccumulation tests. In the present study we measured the uptake (28-d exposure) of polychlorinated biphenyls (PCBs; total and selected congeners) from field-contaminated sediment in the oligochaete *Lumbriculus variegatus*, the mayfly nymph *Hexagenia* spp., and the fathead minnow *Pimephales promelas*. Depuration (25 d) of PCBs was measured in organisms that had been transferred to clean sediment and water after the 28-d exposure. Uptake and elimination of PCBs was rapid in both *L. variegatus* and *Hexagenia* spp. and tissue residues reached steady-state concentrations within 28 d. Elimination rates and biota-sediment accumulation factors (BSAFs) of the PCB congeners were not correlated with  $K_{OW}$  in these invertebrates. Uptake and elimination rates of PCBs were slower in *P. promelas* and it is not clear whether steady-state was reached in the tissues of fish. Elimination rates of the PCB congeners significantly decreased with increasing  $K_{OW}$  in fish. The present study confirms the appropriateness of a 28-d exposure for measuring steady-state concentrations in tissue of the invertebrates, but further study is required for fish.

**TP208 Bioaccumulation of PCBs across Concentration Gradients in Sediments** L.P. Burkhardt, USEPA, Mid-Continent Ecology Division; D.R. Mount, US Environmental Protection Agency, ORD; T.L. HighLand, US-Environmental Protection Agency, NHEERL, MED; J.R. Hockett, US-Environmental Protection Agency, NHEERL, MED; T.J. Norberg-King,

US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; N. Billa, US-Environmental Protection Agency, NHEERL, MED; S.B. Hawthorne, Univ of North Dakota, Energy & Environmental Research Ctr; D.J. Miller, C.B. Grabanski, Univ of North Dakota, Energy and Environmental Research Center. Sediment bioaccumulation tests with *Lumbriculus variegatus* quantify the relationships between the chemical residues in sediments and benthic invertebrates, and these relationships are expressed as biota-sediment accumulation factors (BSAF). At some field sites, BSAFs decrease slightly with increasing concentrations of the chemicals in the sediment, and this behavior has been attributed to the presence of black carbon in the sediment. In this study, 28-day sediment bioaccumulation tests were performed with PCB contaminated sediment samples from Fox, Hudson, and Grasse Rivers Superfund sites. Sediments tested had widely varying total PCB concentrations (0.5 to 150 ppm), total organic carbon contents (0.5 to 25%), black carbon contents (0.1 to 4.4%), and freely dissolved chemical concentrations (measured using polyoxymethylene (POM) coupons) in the sediment interstitial water (0.02 to 65 ng/L). For PCB 118 (2,3',4,4',5-pentachlorobiphenyl), the slopes of geometric mean regressions of the log transformed concentration in the *L. variegatus* ( $\mu\text{g/g lipid}$ ) against log transformed concentration in the sediment ( $\mu\text{g/g organic carbon}$ ) were 0.87 ( $\pm 0.16$ ,  $n=10$ ) and 1.10 ( $\pm 0.14$ ,  $n=10$ ) for the Fox and Hudson Rivers, respectively, and neither slope was significantly different from 1.0 ( $\alpha=5\%$ ). Slopes less than 1.0 imply that the BSAF decreases with increasing concentration in the sediment. Similarly, the slopes of geometric mean regressions of the log transformed concentration in the *L. variegatus* ( $\mu\text{g/g lipid}$ ) against log transformed concentration in the sediment interstitial water were 0.99 ( $\pm 0.07$ ,  $n=10$ ) and 0.78 ( $\pm 0.06$ ,  $n=10$ ) for the Fox and Hudson Rivers, respectively, and only the slope of the Hudson River regression was significantly different from 1.0 ( $\alpha=5\%$ ). Slopes less than 1.0 imply less accumulation in the *L. variegatus* with increasing concentration in the interstitial water. The BSAFs for PCB 118 ranged from 1.3 to 11.7 and from 0.8 to 2.7 for the Fox and Hudson Rivers, respectively. Assuming  $K_{lipid} = K_{ow}$ , prediction of residues in the *L. variegatus* from the measured concentrations in the interstitial water resulted in a median ratio of 0.11 predicted to measured residues for tri-through hepta-PCB congeners ( $n=801$ ). Potential influences of black carbon on accumulation are discussed, along with the implications of this study for predicting chemical residues in the *L. variegatus*. This abstract does not necessarily reflect USEPA policy.

**TP209 Bivalve Shells as Long-term Archives of Trace Metals: The Autoregression Approach** W.A. Shoults-Wilson, Roosevelt Univ, Biological, Chemical and Physical Sciences; L. Seymour, Univ of Georgia, Statistics; J. Unrine, Univ of Kentucky, Plant and Soil Sciences; M. Black, Univ of Georgia, Environmental Health Sciences. Past attempts to utilize the annual rings of bivalves as long-term archives of environmental conditions have met with mixed success. While intensive spatial resolution of trace metal concentrations is possible, it can then be difficult to match this data to specific dates and thus validate by environmental monitoring. In this study, we used laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS) to analyze trace elements (Cd, Cu, Mn, Pb and Zn) on a transect perpendicular to the growth annuli of the freshwater bivalve *Elliptio hopetonensis*. Concentrations of Mn from multiple shells at each site were correlated and average Mn data series were calculated. Periodicity of Mn data was determined and sampling errors removed using an autoregression model. The data series at each site exhibited regular periodicity of high and low concentrations. Periods correlated between the shells from within the same site but were not correlated between shells from different sites. Using Mn correlations to offset Pb series from the same site aligned Pb peaks from multiple shells. This study demonstrates that Mn deposition in the shells of *E. hopetonensis* is a regular, seasonal process. However, this process differs in rate between sites with different environments. It also demonstrates that Mn series can be used to align Pb peaks between multiple shells. This suggests that by using autoregression models to remove sampling error, bivalve shells have the potential to be used as archives of both essential (i.e., Mn) and potentially toxic (i.e., Pb) trace elements uptake by the organism.

**TP210 Comparative Pharmaceutical Metabolism with Rainbow Trout (*Oncorhynchus mykiss*) S9** K.A. Connors, Baylor Univ, Institute of Biomedical Studies, Baylor Univ, Dept of Environmental Science; B. Du, Baylor Univ, The Institute of Ecological, Earth, Environmental Sciences, Baylor Univ, Dept of Chemistry and Biochemistry; Dept of Environmental

Science; P.N. Fitzsimmons, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; C.K. Chambliss, Baylor Univ, Dept of Chemistry and Biochemistry; J.W. Nichols, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; B.W. Brooks, Baylor Univ, Environmental Science and Biomedical Studies, Baylor Univ, Dept of Environmental Science. The occurrence of pharmaceuticals in the environment represents an area of emerging concern. Chemical and biological properties of therapeutics present unique challenges to ecological risk assessment. An important component of risk characterization is understanding bioaccumulation of a chemical. Unlike other industrial chemicals, existing pharmaceutical safety data and pharmacology information may be leveraged through biological "read-across" to aid environmental assessments. However, few approaches and robust empirical datasets exist, particularly for comparative pharmacokinetic applications. For many pharmaceuticals, the primary CYP enzymes responsible for their metabolism have been identified in humans. The purpose of this novel study was to employ a comparative metabolism approach to determine whether rainbow trout possess similar cytochrome P450 (CYP) isozyme-type activity as humans. Test compounds were selected based on their known metabolism in humans by CYP3A4, CYP2D6, or CYP2C9. Metabolism by rainbow trout liver S9 fractions was evaluated using a substrate depletion approach which provides an estimate of the intrinsic clearance rate (CL<sub>INT</sub>). An isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) method was employed for quantitation of parent chemical concentrations. Initial studies suggested that trout possess a limited ability to metabolize the putative CYP3A4 substrates diltiazem and carbamazepine. This observation is important given the accumulation of diltiazem and carbamazepine previously reported from fish in the field by our group. Further results from this effort will help inform the advantages and limitations of cross-species extrapolations of comparative metabolism and pharmacokinetics during risk assessments of pharmaceuticals.

**TP211 Development of an Abbreviated In Vivo Fish Bioconcentration Test** D. Hala, Univ of North Texas, Dept of Biological Sciences; T. Springer, Wildlife International Ltd.; D.B. Huggett, Univ of North Texas, Dept of Biological Sciences. Laboratory bioconcentration studies are becoming an integral part of global hazard and risk assessment paradigms. Experimental measurement of the bioconcentration factor (BCF) is often required for any chemical with log KOW greater than 3, if it is produced in quantities greater than 100 t/year. Currently, the expectation is that a BCF will be experimentally determined for any such chemical by performing a fish bioconcentration study according to Organization for Economic Cooperation and Development (OECD) test guideline 305 or US Environmental Protection Agency (USEPA) guideline 850.1730. These standardized guideline studies are demanding of resources and require the use of large numbers of animals. In an attempt to develop an in vivo screening level test, common carp were exposed to chlorpyrifos, methoxychlor, DDT or musk xylene for 7 d, at which point fish were placed in chemical free water for 7d. During this period, liver and muscle tissues were sampled on days 1, 3, 7, 10 and 14. 14 d BCF values for all chemicals were similar to those values obtained using standard protocols and timelines, suggesting that a reduced design may be helpful in screening chemicals that warrant a more robust bioaccumulation assessment. Statistical and modeling approaches will be applied to these data sets to optimize the experimental design for a robust screening test.

**TP212 Development of In Vitro Assays for Assessing Bioaccumulation Potential of Commercial Chemicals** F. Chen, National Univ of Singapore; B.C. Kelly, X. Liu, S. Bayen, National Univ of Singapore, Dept of Civil and Environmental Engineering. The Stockholm Convention on Persistent Organic Pollutants (POPs) aims to reduce or eliminate the production of persistent, bioaccumulative and toxic (PBT) chemicals. PCBs and DDTs are well-established PBT chemicals, which can cause reproductive failures and cognitive dysfunction in wildlife and humans. Brominated flame retardants (BFRs) and perfluorinated chemicals (PFCs), which have likewise emerged as potentially hazardous PBT chemicals. There is a current need to develop effective in vitro and in silico tools for identifying commercial chemicals that can persist in the environment, bioaccumulate in food chains and cause adverse effects in wildlife and humans. The main objective of the present study was to develop in vitro assays for assessing the toxicokinetics and bioaccumulation potential of commercial chemicals. In particular, we developed a Parallel Artificial Membrane Permeability Assay (PAMPA) to assess passive, transcellular permeability of lipophilic organic chemicals. We also developed

in vitro hepatic microsomal assays utilizing microsomes isolated from liver tissue of two tropical marine fish species (Tiger grouper and Coral trout) to assess metabolic transformation of organic contaminants in marine fish. Test chemicals included several polychlorinated biphenyls, organochlorine pesticides and synthetic musk compounds. The developed PAMPA assays, which involved 96 hour controlled chemical transport between stationary donor and acceptor PDMS disks in solution, were successfully used to quantify apparent permeability (LogPapp) of test compounds. Observed LogPapp values were negatively correlated with chemical Log Kow, consistent with previous reports. The developed in vitro hepatic microsomal assays were successfully used to quantify biotransformation of several PCB congeners and synthetic musks. The extent of chemical biotransformation was variable among compounds and between fish species. Interestingly, Musk Xylene, a nitro musk fragrance compound, exhibited the least amount of metabolic transformation, with only 1-10% of the compound being degraded in these fish liver microsomes. The developed PAMPA and hepatic microsomal biotransformation assays provide a simple and cost-effective approach for eliciting key toxicokinetic data, which can be further incorporated into in silico predictive models to better forecast bioaccumulation potential of commercial chemicals.

**TP213 GIS Based Source Estimation of Cu Pollution in Lake Itzhi-tezhi and Cu Accumulation Profile in *Oreochromis* sp. from the Field and Laboratory Studies** S.M. Nakayama, Graduate School of Veterinary Medicine, Hokkaido Univ, PhD. Student; Y. Ikenaka, Graduate School of Veterinary Medicine, Hokkaido Univ; K. Muzandu, K. Choongo, Univ of Zambia; J. Yabe, T. Muroya, Graduate School of Veterinary Medicine, Hokkaido Univ; S. Ijiri, Faculty of Fisheries Sciences, Hokkaido Univ; T. Umemura, M. Ishizuka, Graduate School of Veterinary Medicine, Hokkaido Univ. [Field study] We reported that concentrations of Cu in sediment and fish liver (*Oreochromis* sp.) from Lake Itzhi-tezhi (ITT) were high, most likely due to the discharge of Cu wastes from the Copperbelt mining area, located 450 km northern of the Kafue River in Zambia. However, it is still not clear whether Cu wastes from the Copperbelt area actually reach Lake ITT. Another question was why *Oreochromis* sp. had higher concentrations of Cu than other fish species. Our objectives were to: (1) clarify the metal distributions in the lake sediment to predict the source of Cu pollution using GIS analysis; (2) reveal if diet is related to Cu accumulation in fish. In this study, GIS analysis suggested that northern part of the lake could be the Cu pollution source. *Oreochromis* sp. showed significantly higher concentrations of Cu than those in other fish species, while concentrations of Cu in the stomach contents among the fish species were not significantly different. [Laboratory experiment] *Oreochromis niloticus* and Japanese medaka (*Oryzias latipes*) were exposed to Cu in order to reveal the species differences in the Cu accumulation or excretion ability. Both species were exposed to Cu (0.2 ppm, waterborne) for 4 days and checked recovery phase up to 14 days. *O. niloticus* showed significantly higher concentrations of Cu compared with medaka at all sampling points. [Conclusions] Northern part of the lake, probably Copperbelt mining area, could be the source of Cu pollution in Lake ITT. Both field and laboratory studies imply that *Oreochromis* sp. contains high concentration of Cu under their physiological condition.

**TP214 Heavy Metal Bioaccumulation in Bivalve Mollusc (*Perna perinea*) in the Coastal Marine Environment Adjacent to ArcelorMittal Tubarão** F. Passamani, ARCELORMITTAL TUBARÃO, Environment, ArcelorMittal Tubarão, Environment; J.O. Santos, ARCELORMITTAL TUBARÃO, Environment. ArcelorMittal Tubarão, one of the biggest world supplier of steel slabs, is located 14km north of Vitória (ES- Brazil). Its domestic and industrial effluents are previously treated and mixed with the sea water used to cool the equipment and structures to be later on thrown into the marine environment. Among the several types of pollutants in the waste generated by a steel making industry are heavy metals which must be carefully considered as they are not biodegradable, are toxic to man in certain concentrations and can be incorporated by the trophic chain. Besides that, heavy metals have already been detected in analysis of the water, the biota and sediments collected in areas adjacent to place where ArcelorMittal Tubarão effluent is thrown into the sea. Bivalve molluscs, especially mussels, are filtering organisms which accumulate composites present in the environment. Therefore, they are constantly used as pointers in environmental monitoring programs throughout the world as they are widely distributed, sedentary or sessile, present on the first levels of the food chain, relatively tolerant to an extensive range of environmental conditions, filter large



volumes of water and bio-accumulate several composites in their tissues. The present study chose one species of mussel to be used as bio-monitor of heavy metals; the *Perna perna* was chosen for meeting all the requirements above, which qualifies the species as a good bio indicator of the area. Thus, the concentrations analysed from 1998 to 2009 for each metal were compared with other works carried out in Brazil and other countries, using the *Perna perna* species as bio-monitor, and classified the areas in two categories: natural or slightly contaminated and contaminated.

**TP215 Occurrence and Bioaccumulation of Pharmaceuticals in a Wastewater Impacted Watershed** B. Du, Baylor Univ, The Institute of Ecological, Earth, Environmental Sciences, Baylor Univ, Dept of Chemistry and Biochemistry; Dept of Environmental Science; J. Berninger, Baylor Univ, Institute of Biomedical Studies; K.A. Connors, Baylor Univ, Institute of Biomedical Studies, Baylor Univ, Dept of Environmental Science; S.B. Junot, T. Conry, Waco Regional Water Quality Laboratory; K. Chambliss, Baylor Univ, Dept of chemistry and biochemistry; B.W. Brooks, Baylor Univ, Environmental Science and Biomedical Studies, Baylor Univ, Dept of Environmental Science. Trace level of pharmaceuticals, considered as contaminants of emerging concern, have already been identified in various environmental matrices globally. Effluent-dominated systems in rapidly urbanizing watersheds represent the worst-case scenarios for waterborne exposure to human pharmaceuticals. Evaluation of environmental exposure and impacts of active pharmaceutical ingredients (APIs) is critical for performing an appropriate assessment of ecological risk for effluent dominated aquatic systems. A recently developed isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology was applied to examine 23 target human and veterinary pharmaceuticals, several metabolites, and other effluent associated contaminants (e.g., sucralose) in surface water samples collected throughout the North Bosque River watershed of central Texas, which receives discharges from six municipal wastewater treatment plants and is severely impacted by dairy Confined Animal Feeding Operations. Surface water concentrations ranged from 0.38 ng/L for diphenhydramine to 3.5 µg/L for sucralose. During dry periods, such as the record setting drought of 2011, stream flow of this watershed is effluent dominated, particularly in the upper reaches of the river basin. Subsequently, we examined bioaccumulation of these target analytes in fish collected from downstream from the first effluent discharge. We furthered measured and predicted plasma tissues and bioconcentration factors (BCFs), based on actual concentration in organisms' tissue and plasma, water concentration of target analytes and accounted for site-specific pHs, which are important to consider during ecological risk assessments of ionizable pharmaceuticals.

**TP216 PCB Spatial Patterns in San Francisco Bay Forage Fish** R.M. Allen, B.K. Greenfield, San Francisco Estuary Institute. Forage fish are frequently employed as biosentinels to evaluate mercury contamination, but spatial surveys of other pollutants are less common. The San Francisco Estuary Regional Monitoring Program (RMP) analyzed polychlorinated biphenyls (PCBs) in forage fish to determine spatial patterns in PCB food-web uptake. Topsmelt (*Atherinops affinis*) or Mississippi silverside (*Menidia audens*) were sampled at 17 randomly chosen sites throughout San Francisco Bay and 12 targeted sites near historically polluted locations. The average sum of 40 PCB congeners in fish from targeted stations (357 ppb, SD = 339) was significantly higher than probabilistic stations (115 ppb, SD = 78). In probabilistic stations, concentrations in topsmelt (154 ppb, SD = 69) were higher than silverside (75 ppb, SD = 68), likely due to elevated lipid content in topsmelt. Both species concentrations were comparable to those of high lipid sport fish in the Bay, and well above a local regulatory threshold for forage fish (10 ppb). Fish PCB concentrations followed spatial patterns in sediment contamination. The highest concentration samples were from targeted Central Bay locations, including Hunter's Point Naval Shipyard (1130 ppb; topsmelt), Stege Marsh (970 ppb; silverside), Oakland Inner Harbor (590 ppb; topsmelt), and Richmond Inner Harbor (340 ppb; topsmelt). Some targeted sites exhibited distinct congener profiles, suggesting local source contributions. These findings indicate that spatial patterns in PCB food web exposure correlate with historical sediment contamination due to industrial activity.

**TP217 Seasonal Changes in the Oxidative Stress Indicators and Heavy Metal Levels in Macroalgae of the Genus: *Gracilaria* in Bahia Magdalena, B.C.S., Mexico** P.T. Rodriguez, Centro de Investigaciones Biológicas del Noroeste, S.C., Laboratorio de Biomedicina y Salud Ambiental; L.M.

Rodriguez, E.S. Zaragoza, T.Z. Savin, Centro de Investigaciones Biológicas del Noroeste, S.C. Marine organisms, such as algae, have been successfully used as sentinels in evaluating the health status of aquatic ecosystems. *Gracilaria vermiculophylla* and *G. textorii* are red algae that are common and abundant in Bahia Magdalena, Mexico. Due to their distribution and ability to accumulate heavy metals in their tissues, they can be used as indicators in environmental health studies. These algal species are an important constituent of the diet of many herbivorous animals influencing their chemical element content with potential consequences in their health. In this study, we compared the levels of Zn, Cd, Cu, Pb and Fe, as well the activities of four antioxidant enzymes: superoxide dismutase, glutathione reductase, glutathione peroxidase, and glutathione S-transferase, and two indices of oxidative damage lipid peroxidation (TBARS) and protein carbonyl levels in the algae *G. vermiculophylla* and *G. textorii*. Macroalgae were collected at Bahia Magdalena, México, in November 2009, and February, May and June, 2010. Seasonal variability of heavy metal concentrations and antioxidant enzyme activities was observed in both species. The metal distribution pattern in marine algae was as follows: *G. vermiculophylla*- Fe > Zn > Pb > Cu > Cd; *G. textorii* Fe > Zn > Cd > Pb > Cu. Both species showed, in general, higher enzymatic activities and lower TBARS and carbonyl protein levels in June, when Pb concentrations were higher. Lower enzymatic activities in algae were found in the seasons with lower Pb, Cd and Cu content. No trend was found for levels of Zn and Fe. This study provides information on the seasonal variations of heavy metal concentrations and oxidative stress indicators in two species of the genus *Gracilaria*, and its potential as sentinel in monitoring marine ecosystems.

**TP218 Translating from Passive Sampling and Passive Dosing to Lipid Based Concentrations in Organisms** P. Mayer, S.N. Schmidt, Aarhus Univ, National Environmental Research Institute; K. Maenpaa, M. Lepanen, Univ of Eastern Finland, Dept of Biology; M.S. McLachlan, A. Jahnke, Stockholm Univ, Dept of Applied Environmental Science (ITM). Equilibrium sampling into various formats of the silicone polydimethylsiloxane (PDMS) is increasingly used to measure the exposure of hydrophobic organic chemicals in environmental matrices, and passive dosing from silicone is increasingly used to control and maintain their exposure in laboratory experiments. Both these equilibrium partitioning approaches are normally calibrated to freely dissolved aqueous concentrations (C<sub>free</sub>), which often are considered the effective concentration for partitioning, bioconcentration and toxicity. In the present studies we extend the calibration of such methods towards equilibrium partitioning concentrations in lipids (Clipid, partitioning). Three case studies will be presented that complement each other: (1) Equilibrium in tissue sampling in three different fish yielded lipid based PCB concentrations in good agreement with those determined using total extraction and lipid normalization. These results support the validity of the in tissue sampling technique, while at the same time confirming that the fugacity capacity of these lipid-rich fish tissues for PCBs was dominated by the lipid fraction. (2) Equilibrium sampling of PCB contaminated lake sediments with PDMS coated vials and with Head Space Solid Phase Microextraction (HS-SPME) yielded lipid based concentrations that were in good agreement with each other. These measurements were about a factor of two higher than actually measured lipid-normalized concentrations in native sediment larvae. (3) Passive dosing was applied to bioconcentration and toxicity studies of several PAHs with the terrestrial springtail *Folsomia candida*. Within the bioconcentration study, equilibrium partitioning concentrations in lipids served as a well defined reference for the evaluation of measured concentrations in the springtails. In the toxicity tests of naphthalene, phenanthrene and pyrene, lethal concentrations were determined also on a Clipid, partitioning basis and were in good agreement with the typical range of lipid membrane burdens for baseline toxicity (40-160 mM). This demonstrates that these new calibration principles also can be applied within a toxicological context.

**TP219 Biodegradable and Low Antimicrobial and Antifungal Toxicity Chiral Ionic Liquids** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences; M. Ghavre, Dublin City Univ, School of Chemical Sciences and National Institute for Cellular Biotechnology. Synthesis of enantiomerically pure compounds is of great importance in organic chemistry. Especially in the pharmaceutical industry the synthesis of chiral pharmaceuticals as a single enantiomer by the most economical method is desirable. Hence various methods, including asymmetric synthesis, the chiral pool strategy, classical



resolution and either kinetic or dynamic kinetic resolution may be employed to obtain an enantiopure product. Use of chiral ionic liquids as solvents for asymmetric synthesis is a new method to obtain enantiomerically enriched products. ILs are often described as 'green solvents'; mainly due to their negligible vapour pressure. However, assessment of their environmental impact is also a requirement, ILs must not persist in the environment and should be non-toxic or of limited toxicity to the environment and all life within. This is a challenging goal for ionic liquid research today. In our present work a library of CILs has been synthesized from methyl imidazole and readily available chiral starting materials in three steps followed by anion exchange. A range of different anions were incorporated into the CILs in order to modulate their physical, chemical properties. When the CILs were tested against seven strains of bacteria they generally displayed low toxicity, and also exhibited favourable biodegradability in a CO<sub>2</sub> headspace test.

**TP220 Green Chemistry Principles Applied to the Synthesis of Novel Opiate Analogues** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences; J. Phopase, Dublin City Univ, School of Chemical Sciences and National Institute for Cellular Biotechnology. The constant pressure to prepare compounds in a more efficient manner has placed the process by which traditional synthetic chemistry is conducted under scrutiny. Pharmaceutical companies desire short, efficient and environmentally friendly synthetic methods. One way that offers this prospect is the minimization of the use of protecting groups in synthesis. A protection/deprotection strategy introduces at least two steps, incurring costs of reagent and waste disposal, and leads to a reduced overall yield. We herein wish to report our short two step synthetic approach with high yields leading to diverse library of novel analgesic compounds. Binding data to the mu opioid receptor is also presented.

**TP221 Green Friedel-Crafts Reaction of Aromatic Compounds with Glyoxamides** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences. The Friedel-Crafts reaction of aromatic compounds with carbonyl compounds is one of the fundamental reactions in organic chemistry. Numerous examples of addition reactions of aromatic compounds to activated carbonyl and alpha-dicarbonyl compounds, such as glyoxylate, trifluoropyruvate, and phenylglyoxal, in the presence of Lewis acids and organocatalysts are known. It provides a simple procedure for the preparation of optically active mandelic acid derivatives, which are important building blocks for many biologically active compounds. Herein, we present the catalytic Friedel-Crafts reaction of aromatic compounds including dimethylaniline to a series of glyoxamides catalyzed by various catalyst systems. We found that the reaction led to the formation of double addition products in the presence of Lewis acid catalysts. Further study of the Friedel-Crafts reaction between N-methylpyrrole and glyoxamides in aqueous NaHCO<sub>3</sub> demonstrated that the reaction selectively produced mono-addition products. Our work provides a 'greener' synthesis of bioactive  $\alpha$ -hydroxyamides and  $\alpha$ -hydroxyamino cores via green Friedel-Crafts type methodology. A detailed study including expanded reaction scope, catalyst optimization and solvent effect will be presented.

**TP223 Ain't No Mountain High Enough: Persistent Organic Pollutants in Remote Irish Lake Catchments** H.E. Scott, Trent Univ, Environmental & Life Sciences, Trent Univ, Environmental and Life Sciences; J. Aherne, C.D. Metcalfe, T.L. Metcalfe, Trent Univ. Long-range atmospheric transport of persistent organic pollutants (POPs) has become an increasing concern on a global scale as evidenced by the enactment of the Stockholm Convention on Persistent Organic Pollutants in 2001. This concern has been driven by the seemingly ubiquitous nature of POPs and their presence in the most remote locations around the world. Ireland is positioned on the western edge of Europe and assumed to be 'pristine' owing to the dominant prevailing Westerlies bringing in 'clean' Atlantic air. As such, it may be considered as a 'background' or reference environment to other European regions. During 2009, POPs were measured in six remote lakes suited around the circumference of Ireland using Semi-Permeable Membrane Devices (SPMDs). The headwater lake catchments were located in high elevation regions (500 to 800 meters above sea level) to avoid influences from local emissions. All study sites were situated in close proximity to the north Atlantic Ocean and Irish sea, thus, receiving inputs solely via rainfall (1200-2200 mm). As anticipated, the concentrations of POPs were low at each of the study lakes. Nonetheless, polychlorinated biphenyls (PCBs) were present across all of the sites, particularly those bordering the

west coast. Additionally, several types of organochlorine pesticides, including Endosulfan, were detected in some or all of the lakes. Concentrations of dichlorodiphenyltrichloroethane (DDT) were highest at sites on the east coast of Ireland possibly due to the influence of national sources or contributions from eastern European airsheds. Moreover, benzene hexachloride (BHC) concentrations were highest in the lake catchment located on the northwest coast. Notably, polybrominated diphenyl ethers (PBDEs), recently listed under the Stockholm Convention annex, were also identified at a number of the study lakes. In general, the lakes were dominated by lighter POPs, more prone to long-range atmospheric transport. Despite the low concentrations measured, the lakes have received a wide range of contaminants from national and 'global' emission sources.

**TP224 An Overview of the Programs Implemented to Assess Shore-line Impacts as a Result of the MC252 Incident** C. Fay, BP, Gulf Coast Restoration; G. Harmon, Cardno Entrix. This poster will describe the studies implemented to determine the spatial extent and degree of impacts of MC252 Incident on the GOM shoreline habitats. In response to the Deepwater Horizon Oil Spill, three programs were implemented to delineate the spatial extent of shoreline oiling in the Gulf of Mexico (GOM): Shoreline Cleanup Assessment Teams (SCAT) overseen by Response organization; rapid pre-assessment mapping by Shoreline Natural Resource Damage Assessment (NRDA) teams; and pre-assessment point evaluation by NRDA teams. The following activities were implemented to assess impacts to shoreline habitats. These programs include: coastal wetland vegetation sampling and monitoring; marsh recession; high-resolution aerial imagery; inter-tidal sediment and soil sampling. These studies will be used to determine the amount of restoration to offset the impacts from the MC252 Incident.

**TP225 Assessment of Perfluorinated Compounds in Fish from US Rivers** L.L. Stahl, J.B. Wathen, USEPA Office of Water, Office of Science and Technology; A.R. Olsen, USEPA, Office of Research and Development, Western Ecology Division, ORD/National Health and Environmental Effects Laboratory; B.D. Snyder, Tetra Tech, Inc., Center for Ecological Sciences; H.B. McCarty, CSC. EPA's Office of Water and Office of Research and Development are collaborating to conduct a national study of fish contamination in US rivers, which includes an assessment of contaminants of emerging concern (CECs) in urban waters for potential impacts to human health. The CEC assessment includes analysis of perfluorinated compounds (PFCs) in fillet tissue from fish composite samples collected at about 150 urban river locations (>5th order based on 1:100,000-scale Strahler order) throughout the lower 48 states. This national-scale urban river study is EPA's first broad assessment of CECs in fish using a statistically-based sampling design. It is being conducted under the framework of EPA's National Rivers and Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the Nation's streams and rivers. The 150 urban river sites form a statistical subset of the 900 sampling locations selected for the NRSA. Samples were collected for the study in 2008 and 2009 during June through October sampling seasons. Field teams across the country applied consistent methods to collect one fish composite sample at every sampling location. Each composite sample consisted of five similarly-sized adult fish of the same species, and the field teams targeted species that are commonly consumed by humans. Largemouth and smallmouth bass were the primary species collected for the study, accounting for 34% and 24% of all fish composites, respectively. Fillets from fish composite samples were analyzed for 13 PFCs, including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). PFCs, and PFOA and PFOS in particular, are heat stable chemicals that repel water and oil, and are commonly used in stain repellants, non-stick cookware, and a variety of other industrial and commercial applications. They are very persistent in the environment and in biota where they bind to blood proteins. Analytical results from this study of US urban rivers indicated that 6 of the 13 target PFCs were detected in fish fillet composites. Frequency of occurrence was dominated by PFOS, which was detected in 76% of all samples.

**TP226 Assessment of Polybrominated Diphenyl Ethers in Fish from US Lakes and Reservoirs** L.L. Stahl, USEPA, Office of Water, Office of Science and Technology, Office of Science and Technology; A.R. Olsen, USEPA, Office of Research and Development, Western Ecology Division, ORD/National Health and Environmental Effects Laboratory; B.D. Snyder, Tetra Tech, Inc., Center for Ecological Sciences; L. Walters, CSC. The US Environmental Protection Agency's Office of Science and Technology

(within the Office of Water) and Office of Research and Development collaborated to conduct a national study of a broad suite of contaminants in freshwater fish. This collaborative effort included analysis of polybrominated diphenyl ethers (PBDEs) in fillet and whole-body tissue samples from lakes and reservoirs throughout the US as part of the National Study of Chemical Residues in Lake Fish Tissue (or National Lake Fish Tissue Study). This was the first national freshwater fish contamination study with sampling sites selected according to a probabilistic (random) sampling design, and it included data on the largest set of persistent, bioaccumulative, and toxic (PBT) chemicals studied in fish to date. An unequal probability design was used to initially develop national estimates for 268 PBT legacy chemicals in fish tissue from lakes and reservoirs of the conterminous United States (excluding the Laurentian Great Lakes and Great Salt Lake). The National Lake Fish Tissue Study also provided the first opportunity to determine PBDE levels in fish tissue on a national basis. Sampling teams collected two five-fish composites from 500 randomly selected lakes throughout the country: a predator (e.g., bass or trout) and a bottom-dweller (e.g., carp or catfish). About one-third of the fillet composites from predator samples and whole-body tissue composites from bottom-dweller samples (which consisted of a statistical subset of samples from 166 of the 500 lakes) were analyzed for 46 PBDE congeners using EPA Method 1614: Brominated Diphenyl Ethers in Water, Soil, Sediment, and Tissue by HRGC/HRMS. Six of the 46 PBDE congeners (PBDE 10, 12, 13, 30, 35, and 166) were not detected in any of the samples. Percent occurrence was dominated by eight congeners (PBDE 28, 33, 47, 49, 99, 100, 153, and 154), accounting for 95% of the total PBDEs measured in the samples. Congener 47 alone accounted for more than 50% of the total detected PBDEs. Total PBDE concentrations were higher in bottom-dweller whole body composites than in predator fillet composites, and maximum concentrations were 125,070 and 58,185 ng/kg (ppt) wet weight for the whole-body and fillet samples, respectively.

**TP227 Comparison of Passive Samplers for Monitoring Dissolved Organic Contaminants in Water Column Deployments** M. Perron, Brown Univ, School of Engineering; R.M. Burgess, USEPA, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, USEPA, USEPA; M. Cantwell, USEPA, Atlantic Ecology Division; E.M. Suuberg, Brown Univ, School of Engineering. Nonionic organic contaminants (NOCs) are difficult to measure in the water column due to their inherent chemical properties resulting in low water solubility and high particle activity. Traditional sampling methods require large quantities of water to be extracted and interference from colloids and/or small particles often exists. In recent years, passive samplers have been used to measure dissolved nonionic organic contaminants. The technique relies upon contaminant partitioning between the aqueous phase and the organic polymer of the passive sampler. The sampler accumulates NOCs resulting in greater analytical detection and measured contaminant concentrations in the sampler can then be used to calculate dissolved contaminant concentrations. In North America, several passive sampling materials are commonly used for NOCs, including solid phase microextraction (SPME) fibers, polyethylene devices (PEDs), and polyoxymethylene (POM). In this study, the utility and effectiveness of these sampling materials for monitoring water column concentrations of several contaminants were compared. Samplers were deployed in galvanized cages in Narragansett Bay, RI (USA) and solvent extracted to analyze for contaminants of concern. For each contaminant, sampler-water partition coefficients ( $K_{\text{sampler-water}}$ ) needed to calculate dissolved NOC concentrations from measured passive sampler concentrations were determined in the laboratory. Differences observed between the three samplers and  $K_{\text{sampler-water}}$  values will be presented for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and the emerging contaminant triclosan. Results from this work will provide useful information for monitoring legacy and emerging contaminants in natural waters.

**TP228 Degradation Patterns of High Molecular Weight PAHs Provide Evidence of Photolysis in Surfaced MC252 Crude Oil** L. Cook, Exponent; P. Boehm, Exponent, Environmental & EcoScience Group, Exponent, Environmental Group, Exponent, Inc., Environmental & EcoScience sGroup; R. Barrick, ENTRIX, Inc. Crude oil samples collected on the water surface from the Deepwater Horizon oil spill showed depletion patterns for the 3- and 4-ring parent and alkylated PAHs that varied from the expected PAH losses associated with dissolution, evaporation, and biodegradation. PAHs in MC252 surface oil showed greater levels of compound-specific

depletion with increasing number of aromatic rings and alkylation levels. Increased levels of depletion were most apparent for the 4-ring PAHs with 3 and 4 methyl groups. Depletion was calculated for individual PAHs using the conserved biomarker hopane with comparison to fresh MC252 crude oil. Based on initial observations of the PAH depletion profiles, the hypothesis that exposure to ultra-violet (UV) light increased the depletion of the alkylated 4-ring PAHs benzo[*a*]anthracene/chrysenes and fluoranthene/pyrenes in MC252 (and hence the degradation of the surface oil and tars) oil was tested using available: field oils and tars as well as surface and deep water samples. Weathering of oil slick samples collected by the USCG Rapid Assessment Teams (RAT) during this spill showed depletion profiles for the alkylated 4-ring PAHs consistent with published information of photodegradation patterns. A sample of the MC252 oil collected immediately after surfacing showed PAH profiles consistent with weathering from dissolution alone, eliminating the transport to the surface as the mechanism for the altered depletion profiles. Oil-impacted water samples collected from the 0-1 meter (m) depth range showed indications of photolysis, likely associated with entrainment of surface oil. Water samples ranging in depth from 1 to 1500 m in depth have PAH depletion profiles that are consistent with PAH losses only from dissolution and biodegradation without any effect from UV exposure. The results of controlled, laboratory UV exposure study of MC252 oil confirm the field observations. Thus analysis of available field data and laboratory studies support the hypothesis that UV exposure increased the depletion of the alkylated 4-ring PAHs in surfaced MC252 oil.

**TP229 Determination of Bisphenol A, Alkylphenols and Alkylphenol Ethoxylates in Human Serum Using a Two-step Solid Phase Extraction and GC-EI/MS/MS** L. Kosarac, C. Kubwabo, Health Canada, Environmental and Radiation Health Sciences Directorate. Bisphenol A (BPA), alkylphenols and related ethoxylates are widely used industrial compounds. Lately, BPA, especially, has been the centre of the attention in the media and general public. In order to contribute to filling the gaps with regards to the lack of information on the levels of human exposure to these emerging contaminants, a new method was developed for the analysis of bisphenol A, alkylphenols and related ethoxylates in human serum. The method consisted of a two-step extraction on Florisil and Oasis HLB cartridges. Target analytes' elution was achieved using dichloromethane and acetone in the first step and methanol and acetone for solid phase extraction on Oasis HLB cartridges. Following the concentration and derivatization with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), the final extract was analyzed by gas chromatography-tandem mass spectrometry (GC/EI-MS/MS). The following compounds were analyzed in this study: bisphenol A, 4-nonylphenol (technical branched isomers mixture, NP), 4-tert-octylphenol (4-(1,1,3,3-tetra-methylbutyl)phenol, OP), 4-n-nonylphenol (linear form), 4-octylphenol (linear form), branched nonylphenol monoethoxylate (NP1EO) and branched nonylphenol diethoxylate (NP2EO). This novel method, unlike many currently available ELISA based methods, offers greater specificity and sensitivity at sub-parts-per-billion levels. Preliminary results show that the concentrations are in the range of 0.936 ng/mL, 10.070 ng/mL, 1.179 ng/mL, 4.030 ng/mL, and non-detected for BPA, NP, OP, NP1EO and NP2EO, respectively. The recoveries for all target compounds ranged between 60 and 115%.

**TP230 Determination of Inorganic Mercury in Fish Liver Using a Direct Mercury Analyzer-80** B. Barst, Université du Québec, INRS-ETE; C. Hammerschmidt, Wright State Univ, Dept of Earth & Environmental Sciences; M.M. Chumchal, Texas Christian Univ, Biology Dept; J. Smith, Univ of North Texas, Dept of Biological Sciences and Institute of Applied Sciences; T. Rainwater, Medical Univ of South Carolina, Dept of Obstetrics and Gynecology, Hollings Marine Laboratory; P. Drevnick, Université du Québec, INRS-ETE. Mercury is found in the environment in elemental, inorganic, and organic forms. Methylmercury, the predominant organic form, compromises the majority of mercury in fish muscle tissue ( $\geq 90\%$ ). For this reason total mercury analysis is often substituted for the more time consuming and expensive methylmercury analysis in fish muscle, while mercury speciation is reserved for analysis of organs like the liver. The percentage of methylmercury in fish liver is more variable and could be dependent on species-specific physiology. Past research from our laboratory and others has linked hepatic damage in fish with elevated hepatic inorganic mercury concentrations. Knowledge of methylmercury and inorganic mercury concentrations is therefore essential when studying hepatic mercury toxicity in fish. Here, we present a quick and relatively inexpensive method

for inorganic mercury determination in fish liver. This method involves microwave acid digestion, removal of the methylmercury from the acid (with toluene) and analysis of inorganic mercury in a diluted aliquot of the acid by Direct Mercury Analyzer (DMA-80). We have demonstrated good reproducibility and recovery of inorganic mercury. Methylmercury can also be analyzed by DMA-80, after it is transferred from toluene to an aqueous phase (L-cysteine solution), but recovery is low (< 70%). Instead, methylmercury concentrations can be calculated by subtracting the determined inorganic fraction from total mercury in the same sample. We are currently validating the method by comparison with accepted techniques (GC-CVAFS and Thiourea Complex IC-CVAFS).

**TP231 Environmental Fate of Decamethylcyclopentasiloxane (D5) Predicted by the Quantitative Water Air Sediment Interaction (QWASI) Fugacity Model** J. Kim, Dow Corning Corporation, Health and Environmental Sciences; D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101); D. Mackay, Trent Univ. Due to the unique characteristic properties of decamethylcyclopentasiloxane (D5), airborne D5 remains in the compartment until it is oxidized by OH radicals. In contrast, when D5 is released to water, multiple processes including volatilization, sedimentation, advection and hydrolysis are expected to take place competitively in the water compartment. Since a better quantitative assessment on the fate, distribution and transport of D5 in a real water body system is needed, this study evaluated the environmental behavior of D5 in the aquatic environment of Lake Ontario at a realistic range of water temperature. Additionally, the assessment included sensitivity and uncertainty analyses to determine the most probable range of the model outcome and the most influential input parameters using a new Excel-spreadsheet version of the Quantitative Water Air Sediment Interaction (QWASI) model. The QWASI model predicts that water concentration of D5 decreases quasi-linearly from 0.31 to 0.05 ng/L due to increase in hydrolysis rate as temperature is increased from 1 to 20°C, whereas sediment concentration of D5 decreases slightly from 1.0 to 0.7 ng/g dry weight. In the same range of temperature, the reaction rate increases from 53% to 93% of the total emission rate while the volatilization rate decreases from 41% to 6%. The effect of changes in rates of sedimentation and advection are relatively small compared with the effects of hydrolysis and volatilization. Both sensitivity and uncertainty analyses indicate that half-life in water and Koc are the most influential input parameters to the outcome of the QWASI model predictions for D5 concentrations in water and sediment. Variances of sediment concentration of D5 and the sedimentation rate are contributed by uncertainties of KOC and half-life in water, whereas variances of water concentration and rates of hydrolysis, volatilization and advection are exclusively influenced by half-life in water. The variance of the model outcome becomes smaller at higher temperature. Thus, the study suggests that Koc and half-life in water as well as emission rate of D5 must be precisely specified in the QWASI model for a better understanding of the fate, distribution and transport of D5 especially in the water environment.

**TP232 Excitation-Emission Matrix Fluorescence Characterization of Dissolved Organic Carbon Leached from Leaves from Various Tree Species** J. Hauri, Assumption College, Natural Sciences Dept; K. Wirzbicki, Assumption College. Dissolved organic carbon (DOC) is an operationally defined parameter that encompasses a variety of chemical structures dependent on the specific allochthonous or autochthonous carbon source. The type of DOC has a significant impact on both the chemical and biological processes that occur in natural waters, as well as the disinfection by-product formation during drinking water treatment. Excitation-emission matrix (EEM) fluorescence spectroscopy has become a more common technique used to characterize the variety of DOC. We investigated DOC leached from different species of leaves collected from trees adjacent to the stream on campus. The leaves were leached in lab using different experimental methodologies to compare the impact of using deionized water versus synthetic moderately hard culture water as the solvent. The samples of leached water containing DOC were analyzed via EEM. Our experimental furthered our knowledge on how DOC derived from different leaves differ from each other, as well as how leaching methods influences the DOC obtained.

**TP233 Field Comparison of Grab Sampling and Time-integrating Passive Sampling Methods for Organic Contaminants in a Freshwater System** J. Crowskrey, J.P. Hassett, R. Oakes, M. Giardono, State Univ of

New York College of Environmental Science and Forestry, Dept of Chemistry. Many hydrophobic organic contaminants (HOCs) exist in aquatic environments at very low concentrations due to low water solubility and high affinity for the particle or gas phase. However, even at low concentrations, many HOCs are potentially harmful to both humans and wildlife due to their persistence in the environment and ability to bioaccumulate. Onondaga Lake in Syracuse, New York is a natural, freshwater lake that is known to have elevated levels of several potentially harmful HOCs, including chlorinated benzenes, polycyclic aromatic hydrocarbons (PAHs), and alkyl benzenes. Due to the potential harm of such contaminants, it is important to accurately monitor their concentrations in freshwater systems like Onondaga Lake and to understand how HOC concentrations may vary with time. Concentrations of several HOCs were measured in Onondaga Lake using passive, in situ, concentration/extraction samplers (PISCES) during the summer and fall of 2009 and 2010. The samplers were filled with hexane and suspended in the water for 7–14 days at three sites. Using an effective sampling rate, the average water concentrations for the compounds of interest were determined during the sampling period at each site. Grab samples were also taken in 2010 for comparison and to refine the effective sampling rate of the PISCES. Significant variation in the concentration of contaminants, in particular the PAHs, was observed during both the 2009 and 2010 field seasons with the passive samplers. Comparison of the passive and grab sample methods shows good agreement for concentrations of most contaminants being monitored, but not for the PAHs. This deviation is due to large, short-term (daily) fluctuations in PAH emissions from a nearby source. The passive samplers capture these fluctuations as composites of the contaminant concentrations during the entire sampling period, whereas weekly grab samples do not reliably capture these fluctuations.

**TP234 Gulf Waters: Protective Enough for Pescetarians?** G. Greenberg, Gradient. In light of the BP oil spill in the Gulf of Mexico, concerns of health risks from fish and shellfish consumption from affected waters have surfaced. In June 2010, the Food and Drug Administration (FDA) released a two step protocol using sensory testing and chemical analyses for reopening federal and state fishers after the BP oil spill. Areas that pass the sensory test are then sampled for fish, shellfish and bivalves. The edible tissue are tested and tissue concentrations are compared against FDA risk based criteria. FDA calculated public health levels of concern using the 90th percentile seafood consumers only consumption rate (13 g/day shrimp and crabs, 12 g/day oysters, 49 g/day finfish) from the 2005-2006 NHANES survey. However, there is a wide array of ingestion rates, which could alter the current FDA levels of concern. In addition, FDA's risk based criteria do not account for the higher ingestion rates among subpopulations (e.g., subsistence populations, native American populations, Asian communities) as well as regions (e.g., Michigan, Louisiana). Consequently, federal and state waters where fish tissue concentrations were below the screening levels may be improperly screened out due to a liberal ingestion rate. Calculations of more conservative ingestion rates and how it could alter FDA's fish residue screening levels will be presented, compared and contrasted with the current standard.

**TP235 Investigation of the Binding of Perfluoroalkyl Acids to Human Serum Albumin Through 19F Nuclear Magnetic Resonance and Fluorescence Spectroscopy** M.P. O'Connor, Union College, Chemistry; L.A. MacManus-Spencer, Union College, Dept of Chemistry. Perfluoroalkyl Acids (PFAAs) are used in the manufacture of a wide variety of consumer and household products, and their presence in the environment is a growing environmental and toxicological concern. PFAAs have been shown to bioaccumulate in the liver and blood stream of many organisms, as well as competing with endogenous ligands when binding to proteins. In this study, the binding of PFAAs to human serum albumin (HSA) over a range of differing pH and ionic strength values was investigated using two experimental approaches. Fluorescence spectroscopy and <sup>19</sup>F nuclear magnetic resonance (<sup>19</sup>F NMR) spectroscopy were used to qualitatively and quantitatively describe the binding of PFAAs with varying chain length and ionic head group to HSA as a function of pH and ionic strength. <sup>19</sup>F NMR experiments were used to probe the binding of PFAAs to HSA by using the distinct signal arising from the fluorocarbon tails of the PFAAs. Fluorescence spectroscopy was used to monitor the native fluorescence of HSA as a function of PFAA binding, monitoring conformational changes in the protein and subsequent changes in the fluorescence spectrum. Fluorescence results indicate that ionic strength (over a physiologically relevant range) has little effect on the binding, while pH has a greater effect. Estimated binding constants from



these studies are on the order of  $10^4 \text{ M}^{-1}$ , varying with fluorocarbon chain length, ionic head group, and pH. The results of this study illustrate the relative effectiveness of these two complementary experimental approaches in estimating PFAA-HSA binding constants and contribute to a better understanding of the role of HSA in the bioaccumulation and pharmacokinetics of PFAAs.

**TP236 Monte Carlo Uncertainty Analysis of a Level III Multimedia Fugacity Model for Decamethylcyclopentasiloxane (D5)** J. Kim, Dow Corning Corporation, Health and Environmental Sciences; D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101); D. Mackay, L. Hughes, Trent Univ. Among several multimedia models, the Equilibrium Criterion (EQC) fugacity multimedia model has been widely used for the assessment of the fate, distribution and transport of a chemical of interest in the environment. Since the model requires partitioning and reactivity properties of the chemical as input data, it is very important to understand how much the uncertainties of the physicochemical properties affect the model outcomes. Thus, this study focused on the uncertainty analysis of the EQC Level III model for decamethylcyclopentasiloxane (D5) to quantify the confidence ranges of mass distribution, persistence and intermedia transport rates based on pre-determined dispersion factors of physicochemical properties of the chemical. For the uncertainty analysis, the Monte Carlo simulation technique was employed using Oracle Crystal Ball as an Excel Add-in program to a new spreadsheet platform of the EQC model. The uncertainty analysis showed that once it is released or vaporized to air, D5 stays in the compartment until it is removed at a relatively fast rate via OH radical oxidation and advection, resulting in relatively short overall persistence and no intermedia transport from air to other compartments. These model outcomes are virtually unaffected by the uncertainties of physical properties of D5. On the contrary, when D5 is released to water, a major fraction is distributed in sediment due to sorption of the compound to organic matter in sediment where the degradation rate is very small. In this case, the model outcome is predominantly influenced by the uncertainty of K<sub>oc</sub>. Under a realistic emission scenario (94.5% to air, 0.8% to water and 4.7% to soil), the 95%-confident ranges of the mass distribution in air and sediment are 47%–78% and 19%–49%, respectively, whereas the overall persistence varies from 3.2 to 5.8 days with the same confidence. The variances are predominantly affected by K<sub>oc</sub>. Overall, the EQC model results are strongly dependent on the mode of emission. Since the mass distribution in sediment is sensitive to the emission to water, accurate measurement of K<sub>oc</sub> and half-life in sediment is expected to afford a better understanding of the fate, distribution and transport of D5 in the environment.

**TP237 New Standard Reference Materials (SRMs) for Environmental Contaminant Analysis** J. Hogue, M. Schantz, National Institute of Standards and Technology (NIST), Analytical Chemistry Division; J.R. Kucklick, National Institute of Standards and Technology (NIST), Analytical Chemistry Division, National Institute of Standards & Technology, Hollings Marine Laboratory; J.M. Keller, S. Vander Pol, R. Pugh, A. Moors, S. Wise, National Institute of Standards and Technology (NIST), Analytical Chemistry Division. For the past 30 years, the National Institute of Standards and Technology (NIST) has developed Standard Reference Materials (SRMs) in response to a growing demand for natural environmental matrices with certified values for organic contaminants. The first environmental SRM issued in 1980, SRM 1580 Organics in Shale Oil, had certified concentrations for five polycyclic aromatic hydrocarbons (PAHs), three phenols, and benzo[*f*]quinoline; however with declining interest in alternative fuel development, SRM 1580 became outdated. In response, NIST has developed a number of environmentally relevant matrix SRMs over the years, including whale blubber, fish and mussel tissues, human blood, house dust, sewage sludge, air particulate material, and a variety of sediments, with a broader range of individual organic contaminants characterized to better suit the needs of the environmental community. Regarding the marine environmental community, NIST has issued several new SRMs, including SRM 2974a Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*), and SRM 1588c Organics in Fish Oil. NIST has also replaced SRM 1941a Organics in Marine Sediment with SRM 1941b and has begun production of SRM 1974c Organics in Mussel Tissue (*Mytilus edulis*), to replace SRM 1974b. These SRMs have been characterized for a suite of organic contaminants, including polychlorinated biphenyl (PCB) congeners, PAHs, polybrominated diphenyl ether

(PBDE) congeners, and organochlorine pesticides. This presentation will summarize the development and certification of these SRMs.

**TP238 Partitioning of Polybrominated Diphenyl Ethers to Dissolved Organic Matter** M. Wei-Haas, The Ohio State Univ, School of Earth Sciences; Y. Chin, The Ohio State Univ, Dept of Geological Sciences; K. Hageman, Univ of Otago, Dept of Chemistry, Univ of Otago. Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame-retardants (BFRs) that are ubiquitous in the environment and undergo long-range atmospheric transport to the Arctic. Recent research indicates that PBDEs, particularly the lower brominated congeners, bioaccumulate and cause liver, thyroid, and neurotoxic effects. The large reported octanol-water partition coefficients (K<sub>OW</sub>) of PBDEs ( $> 10^5$  for tribromodiphenyl ethers to  $> 10^9$  for decabromodiphenyl ether) make these substances highly susceptible to partitioning to dissolved organic matter (DOM). This process would increase the apparent aqueous solubility of PBDEs and alter their transport, bioavailability, and transformation in aqueous environments. The DOM partition coefficients (K<sub>DOM</sub>) for these compounds also vary with organic matter properties such as molecular weight, polarity, and aromaticity. These processes are especially important in the Arctic where PBDE bioaccumulation and phototransformation is highly dependent upon the ability of PBDEs to interact with DOM. DOM partition coefficients for BDE-153 and BDE-99, two commonly detected congeners in environmental samples, were measured by the solubility enhancement method for organic matter collected from Toolik Lake and Oksrukuyik Creek, located on the North Slope in Alaska, and an International Humic Substance Society standard, Suwannee River humic acid. Based on previously derived linear free energy relationships (LFER) between DOM and octanol-water partition coefficients, estimates of log K<sub>DOM</sub> for BDE-153 range from 7.4 to 6.6. Our measured partition coefficients were somewhat lower than the calculated values, with a log K<sub>DOM</sub> of 6.17 and 5.92 for BDE-153 in Suwannee River humic acid and Oksrukuyik DOM, respectively. We attribute these differences to the different types of DOM used in our analysis and those used to establish the LFER. Partition coefficients derived for BDE-153 and BDE-99 will be compared to the composition of each type of organic matter as determined by spectroscopic methods and mass spectrometry as part of our ongoing research examining the role of DOM composition on K<sub>DOM</sub>. We believe that this study will yield insights into both the photolytic fate of PBDEs (via photosensitization reactions with DOM) and their transport, distribution, and bioavailability in the environment.

**TP239 Update to the USEPA's Guidelines for (Human) Exposure Assessment and Monitoring** N.S. Tulye, US Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory; M. Olsen, US Environmental Protection Agency – Region 2, Emergency and Remedial Response Division; M. Broder, US Environmental Protection Agency, Office of the Science Advisor, Risk Assessment Forum. The goal of the USEPA is to protect human health and the environment by understanding, characterizing, and reducing risks to environmental agents. Exposure science evaluates and predicts exposures and provides information for developing exposure and risk assessments as well as the most effective strategies for reducing risks. When conducting a risk assessment, the assessor needs to understand whether an agent may cause an adverse health effect and how exposure to that agent may be reduced. The increasing number and complexity of risk assessments conducted by the Agency presents new challenges. Advances in the field of exposure science require updated resources for conducting exposure and risk assessments. The Guidelines for (Human) Exposure Assessment and Monitoring has been prepared to provide an updated resource for exposure and risk assessors both within and outside the Agency. This document builds on the solid foundation of the 1992 Guidelines, incorporating advances in the field that have occurred since the Guidelines were originally published. It reflects the best science currently conducted across the Agency. This updated document describes the principles of exposure assessment, provides guidance on approaches to conduct an exposure assessment, presents references for more detailed information, and supplies hyperlinks to exposure assessment tools and technical documents. The Guidelines are arranged into chapters, each of which explores a component of the exposure assessment process, including: basic concepts and principles in exposure science; planning and scoping; collecting and using data; using models; planning an observational exposure measurement study; incorporating lifestyles, vulnerable groups, and populations of concern into an assessment; evaluating uncertainty and variability; and presenting and

communicating results. This presentation will highlight and showcase many of the updates in the document.

**TP240 Using Octanol-Air Partition Coefficient (KOA) to Screen Chemicals for Terrestrial Bioaccumulation Potential** M. Kawa, SRC, Inc.; C. Baier-Anderson, US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Design for the Environment Program; R. Boethling, US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Exposure Assessment Branch; M. Citra, SRC, Inc.; J. Costanza, US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Antimicrobials Division; E. Lavoie, E. Sommer, US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Design for the Environment Program; J. Tunkel, SRC, Inc. Bioconcentration factors (BCF) obtained from experimental studies and estimated using quantitative structure activity relationships (QSAR) are widely used to screen chemicals for potential to bioaccumulate in fish. With the introduction of the BCFBAF™ model in EPA's EPI Suite version 4, aquatic bioaccumulation factors (BAFs) can be estimated for three trophic levels of fish using a set of QSARs that incorporate an estimate of the rate of metabolism. A recent investigation (Costanza et al., in press) determined the impact of using upper trophic BAF from BCFBAF™ instead of BCF in screening 4,566 high and medium production volume chemicals for bioaccumulation potential. The results indicate that for nonionic species, most chemicals would retain the same concern level in binning for high/medium/low concern, but approximately 10% of the chemicals would be assigned a higher hazard concern using BAF over BCF. We have extended this work by investigating the potential of chemical substances to bioaccumulate in air-breathing species. It has been suggested that the log octanol/water partition coefficient (Kow) can be used in conjunction with the log octanol/air partition coefficient (Koa) for this purpose, for chemicals that do not metabolize. This work arose from initial results from chemical review under EPA's Design for the Environment (DfE) Alternative Assessment initiative. These chemicals were evaluated using Koa and Kow instead of experimental or estimated BCF, estimated BAF, and available monitoring data. For brominated flame retardant alternatives to decabromodiphenyl ether, bioaccumulation hazard concerns were generally assigned consistently using the predictors described above. For alternatives to Bisphenol A in thermal papers, the concern level for 12 of 14 low bioaccumulation hazard materials could increase using the evaluated Kow and Koa approach, a result that was not entirely consistent with the anticipated metabolism potential of these compounds. We then estimated Kow and Koa for the 4,566 high and medium production volume chemicals evaluated previously with BCFBAF™. A sensitivity analysis of the hazard classifications relative to bright line criteria will be presented, along with the utility of this methodology for screening chemical compounds for potential to bioaccumulate in air-breathing species.

**TP241 Adsorption and Remediation of 17 $\beta$ -estradiol and Bisphenol A Using an Activated Carbon Solution** J. Wang, Univ of Pennsylvania School of Medicine, Teen Research and Education in Environmental Science, Dept of Pharmacology, Center for Excellence in Environmental Toxicology; J. Field, Univ of Pennsylvania School of Medicine, Dept of Pharmacology, Center for Excellence in Environmental Toxicology. The presence and effects of endocrine-disrupting chemicals (EDCs) such as natural and synthetic estrogens on animal and human populations has become a cause for concern not only within the scientific community but also in the public sphere. EDCs present in the environment have caused increased incidences of intersex marine vertebrates and elevated vitellogenin levels in male fish, as well as various malformations and adverse health effects in humans, such as hypospadias and cryptorchidism. It is imperative that methods of filtering or otherwise removing endocrine disruptors be created and implemented. One such promising method is that of activated charcoal filtration. In this study, the bioluminescent yeast estrogen screen (BLYES) developed by Sanseverino et al. was used to determine the estrogenicity of standard and experimental solutions. Experiments using a 10% activated charcoal solution achieved 99.1% and 79.3% reductions, respectively in the concentration of two known natural and synthetic estrogens, 17 $\beta$ -estradiol and bisphenol A. Activated charcoal holds great potential for reusable and highly effective EDC filtration, but more experiments must still be performed to optimize this method and to enact its widespread application.

**TP242 Ambient Thermal Desorption Ionization for Rapid Mass Spectrometric Analysis of Contaminants** G. Cleland, J. Burgess, Waters Corp.;

K. Rosnack, Waters Corporation; D. Shaw, C. Mallet, M. Twohig, Waters Corp. To ensure the safety of consumers following an oil spill, a rapid screening method is required to analyze food for compounds of concern. Of the many compounds found in oil, a subset of major concern is the polyaromatic hydrocarbons (PAHs). These compounds are known to be carcinogenic and the US Environmental Protection Agency has defined these compounds as priority pollutants. The application of ambient desorption techniques for sample introduction into mass spectrometers is an emerging technology that has applicability in many areas of contaminants analysis. The atmospheric-pressure solids analysis probe (ASAP) is a unique mass spectrometry technique for direct analysis of volatile, semi-volatile, solid and liquid samples. In ASAP, samples are introduced to the mass spectrometer on a glass capillary and vaporized with heated nitrogen desolvation gas. Ionization is achieved using a corona discharge. While conventional analytical tools, such as LC or LC/MS, require time-consuming sample preparation procedures, ASAP can provide mass spectra within seconds of sampling. The use of ASAP coupled to a tandem quadrupole mass spectrometer for the detection of PAHs in seafood samples was investigated. Spiked samples of fish and shrimp were homogenized and then sampled directly with the glass capillary. PAHs were screened using multiple reaction monitoring (MRM) mode. In addition, PAHs were extracted from the samples using a simple QuEChERS protocol and the extract sampled directly using the capillary. Results from both approaches will be presented. This work provides an interesting example of the potential applications of ASAP for the analysis of environmental contaminants and will discuss the benefits and challenges of the technique.

**TP243 Comparing Total Hg, 'Reactive' Hg and Speciated Hg in Natural Waters** B. Jackson, Dartmouth College, Dept of Earth Sciences; V. Taylor, Dartmouth College. Here we compare total Hg, determined by BrCl oxidation, SnCl<sub>2</sub> reduction, gold trapping and pyrolysis and AFS detection, reactive Hg, determined as for total Hg but without the BrCl step, and speciated Hg determined by speciated isotope dilution, automated purge and trap-ICP-MS on natural waters collected from ponds, rivers and vernal pools. The water samples were filtered at 0.45  $\mu$ m, 0.2  $\mu$ m and 10KDa to assess the effect of dispersed colloids and nanoparticles on the comparison. We hypothesize that incomplete isotopic equilibrium of inorganic Hg with the particle bound Hg causes underestimation of total dissolved Hg by the speciated isotope dilution method. Furthermore particle-bound inorganic Hg is not SnCl<sub>2</sub> reducible and this operationally defined fraction records the lowest concentration of dissolved Hg.

**TP244 Coupling Asymmetrical Flow Field Flow Fractionation with ICP-MS for Characterization of Silver Nanomaterials in Aqueous Samples** E. Hoque, Trent Univ, E. & R. S.; C.D. Metcalfe, Trent Univ, Environmental & Resource Studies, Trent Univ, Dept of Environmental & Resource Studies. Silver nanomaterials (nAg) are widely used in consumer products, such as textiles, personal care products, food storage containers, laundry additives, home appliances and paints, because of its antibacterial properties. According to a 2009 report from 'The Project on Emerging Nanotechnologies', there are 259 listed consumer products containing nAg. It is likely that nAg enters into the aquatic environments from discharges of domestic and industrial wastewater. Hence, it is important to develop proper analytical tools to quantify nAg in water to assess exposures to aquatic organisms. In this study, we developed an analytical method using asymmetrical flow field flow fractionation (FFF) and inductively coupled mass spectrometry (ICP-MS) to determine the size and concentration of nAg in aqueous samples. Analysis of a mixture of 20, 40, 60 and 80 nm nAg standards suspended in water resulted in a well resolved fractogram. Retention times of nAg were correlated with nanomaterial sizes. The current limit of quantitation of FFF-ICP-MS method is ~ 1.0 ng/mL nAg, which is within the range of predicted concentrations of nAg in wastewater. Using this method, nAg was detected in an untreated wastewater (i.e., influent). The estimated size and concentration of nAg in the influent of a wastewater treatment plant (WWTP) were 10 – 20 nm and ~ 2.0 ng/mL, respectively. This result is being validated by transmission electron microscopy investigation. In addition, the developed method is being utilized to evaluate the fate of nAg in WWTP and characterize nAg in exposure water to support aquatic toxicology studies.

**TP245 Determination of Tamoxifen and its Major Metabolites in Exposed Fish** S. Jayaraman, M. Simoneau, R. Pruett, R. Gutjahr-Gobell,

G. Zaroogian, L. Mills, USEPA. Tamoxifen (TAM), (Z)-1-(p-dimethylaminoethoxyphenyl)-1, 2-diphenyl-1-butene, is a nonsteroidal agent that has been used in breast cancer treatment for decades. Its major metabolites are 4-hydroxytamoxifen (4-OHT), N-desmethyltamoxifen (DMT), and endoxifen. While TAM and metabolites have been measured in humans, rats, and chickens, we know of no studies conducted in fish, although fish are likely to be exposed to these chemicals through wastewater effluent entering aquatic environments. The purpose of this study was to develop and validate an analytical method for quantifying TAM and metabolites in fish plasma. We tested and modified the published analytical methods of Zhu et al. (2008, J Pharmaceut Biomed, 46: 349-355), which use UV exposure to convert TAM and metabolites to fluorescent derivatives for HPLC detection. We evaluated mexiletine, propranolol, methoxytamoxifen, clomiphene and toremifene for internal standards. To determine optimal extraction and analytical methods, plasma samples from untreated fish were spiked with TAM and metabolites. Different solvents for extraction were evaluated, including diethyl ether (DEE) and various hexane/butanol mixtures. We varied combinations of mobile phase to optimize the separation, settling on an 82:18, methanol: 1% aqueous triethylamine solution. The optimal time for photochemical conversion of TAM and metabolites to phenanthrene derivatives, using a 4-watt UV lamp, was assessed. Chromatographic separations were carried out on a Waters HPLC with a 2690 Alliance separation module, 996 photodiode array detector, and 474 scanning fluorescence detector. The column was an Agilent Zorbax Extend C18 maintained at 60°C preceded by a matching guard column. A flow rate of 0.75 ml/minute was found to maximize separation of all analytes. The method detection limit was 1 ng/mL. Cunner (*Tautoglabrus adspersus*) were given a single 25 mg/kg dose of TAM by oral gavage, then approximately 0.5 ml of plasma was drawn from a caudal vein 1, 4, 8, 16, 20, 24, 48 and 72 hours later. Three fish were sampled at each time point, and each fish was sampled twice during the study. The analytical method that we developed is being utilized to evaluate TAM and metabolites in the plasma of these fish.

**TP246 Development and Validation of Silicone Passive Samplers to Examine Oxygenated-PAHs in the Portland Harbor Superfund, OR** S.G. O'Connell, Oregon State Univ, Environmental and Molecular Toxicology; T.A. Haigh, Oregon State Univ, EMT Dept; S.E. Allan, Oregon State Univ, Environmental and Molecular Toxicology, Oregon State Univ, Graduate Researcher; G. Wilson, Oregon State Univ, EMT Dept; L. Tidwell, K.A. Anderson, Oregon State Univ, Environmental & Molecular Toxicology. Passive samplers have been deployed to examine bioavailable fractions of contaminants in many matrices including air, water, and sediments. New deployment devices and materials have been developed in the last decade to sample contaminants with wide ranges of physiochemistry properties, including non-polar and polar organic compounds. Silicone polymers have been increasingly used as passive sampling devices in laboratory and field studies, mostly concentrating on polycyclic aromatic hydrocarbons (PAHs). Due to the intramolecular forces inherent in this material, silicone may preferentially sequester semi-polar organic compounds such as Oxygenated-PAHs (OPAHS) over other polymers used as passive sampling devices. OPAHS are characterized in recent publications as an emerging class of contaminants with environmental concentrations and toxicities similar to those of comparable PAHs. Early work supports our hypothesis by comparing silicone and lipid-free polyethylene tubing using GC-MS Automated Mass Spectral Deconvolution and Identification System (AMDIS). Several semi-polar organic compounds ( $\log K_{ow}$  2.0-4.8) were identified exclusively in the silicone polymer, including 9,10-Anthraquinone at the Portland Harbor Superfund Site, OR. Current work will quantify any additional OPAHS present in the extracts, and the results of these analyses will be compared to those values found in lipid-free polyethylene tubing deployed in tandem at the same field site.

**TP247 Development of a Sample Preparation Procedure for the Determination of 17 $\alpha$ -ethinylestradiol in Whole Fish Tissues** A. Alansari, A. Saleem, L. Kimpe, V. Trudeau, J. Blais, Univ of Ottawa, Biology. Analysis of whole fish tissue extracts containing steroidal estrogens can be a challenging task to accomplish mainly due to the presence of co-extracted lipids and proteins that can hinder the analysis. The purpose of this study was to develop and optimize a targeted method for the sample preparation and analysis leading to a successful determination of 17 $\alpha$ -ethinylestradiol (EE2) in whole fish tissues at ng·g<sup>-1</sup> levels. The procedure includes the extraction of whole fish tissue by pressurized liquid extraction (PLE), followed by lipid

removal using gel permeation chromatography. Then, a crucial acetonitrile precipitation cleanup step was applied to facilitate the removal of cholesterol. The analysis was performed on a gas chromatograph/mass spectrometer (GC/MS) in selected ion monitoring (negative polarity). The method was developed using high lipid content wild fish spiked with EE2 ranging from 12.5-50 ng·g<sup>-1</sup>. The recovery of the analyte ranged from 74.5-93.7% (RSD of 2.3-6.2%). The method detection limit was 0.68 ng·g<sup>-1</sup> dry weight (dw). The developed method was applied to determine EE2 levels in goldfish (*Carassius auratus*) after a 72h dietary exposure. EE2 was detected in all of the seven exposed samples averaging 2.07±0.85 ng·g<sup>-1</sup> with method detection limits of 0.68 ng·g<sup>-1</sup>. Funded by NSERC-Canada and King Abdulaziz Univ, Saudi Arabia.

**TP248 Development of Modified QuEChERS Extraction Methods for the Analysis of PAHs in High Fat Content Fish and Smoked Salmon** N.D. Forsberg, G. Wilson, Oregon State Univ, Environmental and Molecular Toxicology; K.A. Anderson, Oregon State Univ, Environmental & Molecular Toxicology. The QuEChERS extraction methods are popular techniques for extraction of bio-matrices because they are seen as Quick, Easy, Cheap, Effective, Rugged and Safe. However, we found that traditional QuEChERS methods were ineffective at recovering polycyclic aromatic hydrocarbons (PAHs) from high fat fish. Specifically, we demonstrated the poor performance of the traditional method in recovering (often < 60%) a large number of PAHs from fatty salmon samples. To fill this knowledge gap, modified extraction methods were developed and validated for 33 PAHs in smoked salmon with 3-11 % fat content. Sample preparation for the modified methods includes extraction of PAHs into a (2:2:1, v/v/v) solution of ethyl acetate, acetone and iso-octane followed by cleanup with dispersive SPE and analysis by GC-MS in SIM mode. Spike recoveries of some 2, 3 and 5-ring PAHs were improved 50 – 200% over traditional methods, while average recovery across all PAHs was improved 67%. Method precision and sensitivity were good with replicate extractions typically yielding relative standard deviations < 10% and detection limits in the low ng/g range. Results of the current work add to the understanding of QuEChERS sample preparation applications, specifically for extracting hydrophobic contaminants from high fat bio-matrices, and demonstrate limitations of traditional extraction schemes.

**TP249 Diffusive Flux of Nanoparticles Through Porous Membranes with Chemically Modified Surfaces** J.R. Stephens, The Pennsylvania State Univ, Chemistry; M.E. Williams, The Pennsylvania State University, Chemistry. Diffusive transport of nanoparticles through mesoporous materials are examined using a diffusive cell with two compartments separated by either a porous alumina or polycarbonate membrane. The initial experiments were conducted as a function of pore size, particle diameter, and solvent. Rates of transport were monitored by a change in absorbance of nanoparticle solutions in the receiving cell. Flux (J) of 3, 8, and 14 nm diameter CoFe<sub>2</sub>O<sub>4</sub> particles were determined using the measured extinction coefficients and change in absorbance of the solution as a function of time as the particles were translocated across porous membranes with diameters of 30, 50, 100, and 200 nm in hexane and aqueous solutions. In all cases, J was slower through pores than in bulk solution and decreases as pore diameter decreases, however flux is inversely related to particle size. J values were larger in for aqueous solutions than in hexane, which is thought to result from the hydrophilic nature of the unmodified membranes. Pore surface chemistry plays an important and complementary role determining particle flux through pores, and ultimately in achieving selectivity in separation of nanoparticles and nanoarchitectures. These studies have used nanoparticles of 4, 8.5, and 14 nm diameter CoFe<sub>2</sub>O<sub>4</sub> particles and alumina membranes with specific surface chemistries. The membranes are modified by the direct attachment of siloxanes with varying chains of varying length and terminal functionality. The results show that flux is greater when a hydrocarbon chain lines the interior pore wall compared to the initial studies of untreated membranes. Also, by altering the terminal head group of the attached siloxanes, flux of the CoFe<sub>2</sub>O<sub>4</sub> nanoparticles is affected. These experiments allow us to explore the role of intermolecular interactions between the pore and particles and the impact on particle transport.

**TP250 Distribution of Selected Wastewater Derived Pharmaceuticals in the Grand River Watershed** L. Bragg, R. Tanna, Univ of Waterloo, Dept of Biology; G.R. Tetreault, Univ of Waterloo, Biology; M.E. McMaster, Environment Canada, National Water Research Institute; M.R. Servos, Univ of



Waterloo, Dept of Biology and Canadian Water Network, Univ of Waterloo, Dept of Biology. There has been increasing interest in pharmaceuticals and personal care products (PPCPs) in the environment over the past decade as technology continues to advance and detection of more compounds becomes possible. As populations continue to increase these chemicals are continuously added into the river systems since our waste water treatment plants (WWTPs) are not designed to remove PPCPs from the treated effluent. The Grand River watershed hosts a large population (~925,000) which is expected to increase substantially in the next decade. In the Upper Grand River, the major contributing WWTPs are in the cities of Waterloo, Kitchener, and Guelph. Waterloo and Kitchener have only secondary treatment whereas Guelph has tertiary treatment. We have completed a survey of selected emerging contaminants in the river water plus the WWTP effluent to coincide with other fish population and intersex studies completed in 2010. Samples were collected, preserved, transported to the lab prior to extraction with solid phase extraction (Oasis HLB 6cc, 500 mg cartridges). All samples were spiked with isotopically labeled standards to compensate for possible losses and matrix effects in the analysis. Analysis was completed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an Agilent 1200 LC with an ABSciex 3200 Qtrap MS with electrospray ionization in both positive and negative modes. A wide range of emerging contaminants were analyzed such as ibuprofen, carbamazepine, diclofenac, fluoxetine, naproxen and venlafaxine with detection limits all below 10 ng/L. Atrazine, a pesticide used frequently in the watershed, was also monitored in the analysis. The concentration of this pesticide remained relatively consistent throughout the watershed. Pharmaceutical concentrations, on the other hand, varied widely through the watershed increasing downstream of each WWTP. Distinct differences were observed between the effluents of the treatment plants with different treatment processes.

**TP251 Effect Directed Assessment: Evaluating Estrogenic Activity of Municipal Wastewater Effluents** B.M. Smith, K. Oakes, H.E. Engelhardt, L. Bragg, Univ of Waterloo, Dept of Biology; W. Parker, Univ of Waterloo, Dept of Engineering; M.R. Servos, Univ of Waterloo, Dept of Biology. Municipal Wastewater Effluents (MWW) are potential anthropogenic sources of estrogenic substances. Fish exposed to these substances have shown estrogenic responses at various biological levels including estrogen receptor (ER) activity, elevated circulating vitellogenin (Vtg) and the occurrence of intersex within testes. An Effects Directed Assessment (EDA) was applied to three MWW and three municipal wastewater pilot plant effluents to evaluate estrogenic activity using the yeast (*Saccharomyces cerevisiae*) estrogen screen (YES). Each effluent was also analyzed for Vtg using an indirect competitive enzyme linked immunosorbent assay (ELISA) in Rainbow Trout (*Oncorhynchus mykiss*) exposed for 8 or 14 d to each MWW. The MWW represent a range of treatment from conventional activated sludge to tertiary processes that discharge into the Grand River (Ontario, Canada). The pilot plant wastewater effluents represent three different processes: conventional activated sludge (CAS), nitrifying CAS (CAS-N), biological nutrient removal CAS (CAS-BNR). Effluent samples were collected, extracted by solid phase extraction (SPE) and fractionated by high performance liquid chromatography (HPLC) to separate potential estrogenic compounds and assayed for ER agonists using YES. All of the fish exposed to the MWW showed no physiologically significant induction of circulating Vtg within or between treatments. However, preliminary results using YES show the presence of ER agonists in whole effluent extracts. Known estrogenic compounds including the natural hormone estrone have been isolated and identified at biologically active concentrations. Further comparisons among the treatment plant processes and pilot plant effluents are being conducted to elucidate and isolate other potential estrogenic substances.

**TP252 Endocrine Disrupting Compounds and the Horseshoe Crab Populations of Delaware** E.S. Maung, Univ of Delaware, School of Marine Science and Policy; A.K. Aufdenkampe, Stroud Water Research Center; D.C. Miller, Univ of Delaware, School of Marine Science and Policy. The American horseshoe crab, *Limulus polyphemus*, is one of several horseshoe crab species, but the only one found along the coast in North America. With a range from northern Maine to the Yucatan peninsula in Mexico, this species has its highest spawning densities in Delaware and is of great importance to the fishing, biomedical and ecotourism industries. Because horseshoe crabs spend the first 3 weeks to few months of life buried in coastal beach sediments, they are susceptible to endocrine disrupting compound (EDC) exposure from sources such as

runoff and groundwater. Little is known about what endocrine disrupting compounds may accumulate in the tissues of horseshoe crabs, or how those contaminants will impact larval development. Furthering our understanding of these parameters is critical to understanding chemical problems in the Delaware estuaries, as horseshoe crabs hold a key role in the food web and thus in maintaining ecosystem integrity and biodiversity. We took a novel approach to examine these unknowns by modifying a pre-existing gas chromatography mass spectrometry method to identify which EDCs horseshoe crabs accumulate while developing in beach sediments. This method was implemented on eggs, embryos and larvae collected from a total of 6 sites from within the Delaware and Inland Bays. These samples were examined for accumulation of pesticides, surfactants, antimicrobials, as well as estrogens. Nonylphenol and triclosan were among the most ubiquitously found compounds. Alachlor and 17 $\alpha$ -ethinylestradiol had the highest tissue concentrations (0.24 and 0.73  $\mu\text{g/kg}$  respectively). Experiments are currently underway to determine the impact of chronic exposure to these compounds on larval development.

**TP253 Enrichment of Chromium, Nickel and Cobalt in Sediments, Water, Mussels and Fucus Influenced by Natural Mineral Sources** M. Caetano, INRB IP/IPIMAR; R. Prego, N.O. Alvarez, Marine Research Institute (CSIC); J. Raimundo, INRB-Ipimar, Biogeochemistry & Environmental Impact; C. Vale, INRB IP/IPIMAR. Geological complex close to the shoreline could be significant contributors of trace metal to the coastal environment. This is a key question poorly considered that could be blend with anthropogenic contamination events. Therefore, the aim was to research the influence of chromium (chromite and chromospinel) and nickel (gersdorffite and pentlandite) minerals associated with ultrabasic rocks of Cape Ortegal and pirrotites of Herbeira Massif, on its adjacent coastal area of north Galicia. In order to achieve this objective (a) the tributary rivers Mera, Landoi and Lourido that flow from Ortegal hillside to the Ortigueira Ria were fortnightly sampled during 2008; (b) the surface sediment of Northern Galician Rias and their adjacent shelf was sampled in July 2008; (c) samples of mussels and fucus were seasonally collected during 2008 inside the Ortigueira Ria. Dissolved Cr and Ni was analysed by ICPMS while particulate samples and organisms were mineralized in a microwave following EPA procedures and metals analyzed by GFAAS. Levels of Cr in surface sediments ranged 200-930, 230-1340 and 290-1660  $\text{mg}\cdot\text{kg}^{-1}$  in inner and outer ria and shelf, respectively. Results confirm enrichment of Cr, five to forty times, higher than background levels of other Galician rias. Using Mg as a particle tracer it maybe concluded that Ortegal Cape was the Cr source. Moreover, rivers indicate the same pattern: Landoi and Lourido in Ortegal area had 7-48 and 5-81 nM of dissolved Cr and 0.2-0.8 and 0.2-1.3  $\text{g}\cdot\text{kg}^{-1}$  of Cr in SPM while out of Ortegal complex Cr in Mera River was < 0.2nM and < 0.3  $\text{g}\cdot\text{kg}^{-1}$ . In biota, Cr levels (2.0-3.5  $\text{mg}\cdot\text{kg}^{-1}$ ) were similar to mussels of other coastal rias whilst in fucus higher levels were found in Ortigueira (7-16  $\text{mg}\cdot\text{kg}^{-1}$ ). The Ortegal Cape was also identified as the main Ni source using Mg as a particle tracer. Levels of Ni in coastal surface sediments ranged 100-620, 140-470 and 160-1360  $\text{mg}\cdot\text{kg}^{-1}$  in inner and outer ria and shelf of Ortigueira, respectively; these concentrations decreased to 5-160 and 4-87  $\text{mg}\cdot\text{kg}^{-1}$  in the neighbouring rias of Barqueiro and Viveiro. Results pointed to a high sedimentary Ni enrichment, four to twenty times respect to usual rias background concentrations, in the coastal environment of the Ortegal Cape. Fluvial discharges also indicated the same geological source: Lourido and Landoi transport  $190\pm95$  and  $80\pm30$  nM of DNi and  $24\pm17$  and  $95\pm90$  nM of PNi while out of Ortegal complex Mera River had  $15\pm7$  nM of DNi and  $4\pm3$  nM of PNi. In biota, Ni levels (3.4-6.9  $\text{mg}\cdot\text{kg}^{-1}$ ) were similar to mussels of other rias whilst in fucus the highest concentrations were found in Lourido mouth (46  $\text{mg}\cdot\text{kg}^{-1}$ ), decreasing to ria mouth (8.5  $\text{mg}\cdot\text{kg}^{-1}$ ). Coastal enrichment in trace metals can also occur due to natural contributions apart from anthropogenic contamination. It is the observed case of Cr and Ni in the fluvial, estuarine and marine environment close to Cape Ortegal as result of the presence of chromium and nickel minerals.

**TP254 Environmental Fate of Glyphosate in Broom-infested Mt. Tamalpais Soil** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering, Univ of California, Dept of Civil and Environmental Engineering. Glyphosate is a post-emergent and non-selective organophosphorus herbicide, which is the most commonly used in the US for weed control in residential, commercial, agricultural, and forestry settings. The present study investigated environmental decay of glyphosate in surface soil and on the broom leaves as well as lateral and vertical transport

by stormwater runoff and soil infiltration. Environmental half-life in the surface soil was 44 days, while concentrations of glyphosate in broom leaves didn't exhibit significant changes over 84 days of study period. Significant fractions of leaves were still remained on the stems at the end of the study period and thus the slow degradation on leaves needs to be combined together with relatively faster degradation in soils to calculate overall degradation of glyphosate. Glyphosate was not found in two stormwater runoff collected at a site about 50 feet from an application site. Glyphosate was detected in core soils up to 30 cm deep with the highest concentrations in the top layer. Laboratory infiltration simulation study showed that glyphosate can further move down through the soil layers up to 54 cm, though water infiltration rate used for the laboratory study was not realistic.

**TP255 Environmental Quality in Recreational Beaches in Campeche, Mexico** L. Alpuche Gual, Campeche Autonomous Univ/EPOMEX Center, Centro Epomex; M. Lara-Flores, Y. Sulub-Palafox, G. Poot-Cruz, B. Ramirez-Vargas, Campeche Autonomous Univ/EPOMEX Center. Campeche coastal tourism depends on clean beaches, unpolluted water, well-planned coastal areas and coastal and marine ecosystems preserved. Despite the importance that the beaches are in coastal development in the zone, have become the receptacle of contaminated water collected by the urban sewer systems or storm drains – often without treatment – water courses, maritime areas adjacent to their coasts, or even directly to the beach. The aim of this study was to evaluate the environmental quality of coastal waters from several beaches in Campeche, Mexico through the measurement of water quality parameters and determination of pollutants identified by the Mexican standards. Water and sediment samples were collected on a network of sampling sites in five public beaches and adjacent coastal every month during an annual cycle. Using a multiparameter sonde we measured in situ pH, conductivity, temperature, salinity, TDS, LDO and %LDO. The results indicate poor water quality especially in Campeche and Carmen beaches to good in Champoton beaches. Measured levels of faecal coliforms were above the limits established by Mexican law. This, besides a deficiency in dissolved oxygen and a high organic matter concentration (as COD), reflects eutrophication processes in some areas of the coastal zone in Campeche and Carmen. Multivariate analysis was used to regionalize the beaches in Campeche as a function of the behavior of all measured parameters. It was concluded that discharges of untreated wastewater, impact the environmental quality of these shores in some seasons, particularly total and fecal coliforms, which are hazardous to the health of recreational users of these sites.

**TP256 HPLC-ESI/MS/MS Method for the Determination of Endogenous Estradiol and Testosterone in Fish Plasma** M. Fleischer, S. Devellis, Smithers Viscient; R. Biever, Smithers Viscient, Dept of Environmental Toxicology; P. Riebach, Smithers Viscient. Estradiol and testosterone are two key steroids of the endocrine system, which can be measured as optional endpoints in the Fish Short-Term Reproduction Assay. The levels in plasma can be used as an indicator of disruption in endocrine system functions. An HPLC-ESI/MS/MS method was developed and validated for the simultaneous determination of both estradiol and testosterone in fish plasma. Whole blood was collected manually from fathead minnows (*Pimephales promelas*). Plasma was generated by centrifugation. Analyses were performed on small volume plasma samples (generally  $\leq 10 \mu\text{L}$ ). Plasma proteins were removed by either centrifugation or using a 96 well filtration format plate. Estradiol was derivatized with dansyl chloride. Testosterone was determined without modification. Deuterated internal standards for both were utilized. The LOQ for the analysis is 0.3 ppb for estradiol and 0.15 ppb for testosterone. Endogenous levels for control fish generally agreed with published values. This method will be used in conjunction with Tier 1 Endocrine Disruptor Screening Program (EDSP) assays.

**TP257 Metabolic Activation of Halogenated Bisphenol A by Rat Liver S9 and Their Thyroid Hormone Activity** M. Terasaki, Univ of Shizuoka, Institute of Environmental Sciences; Y. Seki, M. Makino, Univ of Shizuoka. The thyroid hormone (TH) agonist/antagonist activities of halogenated derivatives of bisphenol A (BPA) were assessed using a yeast two-hybrid assay incorporating the human thyroid hormone  $\alpha$  (TR $\alpha$ ), both with and without possible metabolic activation by rat liver S9 preparation. In the absence of the rat liver S9 preparation, 3,3',5,5'-tetrabromobisphenol A (TBBPA), 3,3',5,5'-tetrachlorobisphenol A (TCBPA), and 3,3',5-trichlorobisphenol A (3,3',5-triClBPA) exhibited agonist activity, whereas 3-chlorobisphenol A

(3-ClBPA), 3,5-dichlorobisphenol A (3,5-diClBPA), 3,3'-dichlorobisphenol A (3,3'-diClBPA), and BPA did not. In the presence of the rat liver S9 preparation, three halogenated BPAs showed an increase in agonist activity. The activities of TBBPA and TCBPA increased markedly after their metabolic activation with the rat liver S9 preparation (7.6-fold and 3.1-fold, respectively). With regard to the biotransformation of halogenated BPA, TBBPA undergoes oxidative cleavage near the central carbon of the molecule, leading to the production of hydroxylated bromophenol by liver S9 fractions. Several halogenated phenols are capable of acting as TH-like compounds in in vitro assays and are more active than TCBPA or TBBPA. Some yeast strains are also able to degrade dibenzofuran or biphenyl up to ring cleavage in the presence of the co-metabolic substrate. The activity of the metabolites of halogenated BPA may contribute to the elevation of the observed TH activity by exposure to the S9 preparation or yeast strain. TBBPA, TCBPA, and 3,3',5-triClBPA inhibited the binding of triiodothyronine (T3) to TR $\alpha$  at 20,000 nM without rat liver S9 treatment and 4,000 nM with rat liver S9 treatment, demonstrating their T3 antagonist activity. These antagonist activities were enhanced by exposure to S9 metabolic activation at the same concentration level. In this study, we showed the agonist/antagonist activities of some halogenated BPAs, as well as their assumed effects in humans, and their metabolic activities with respect to TH when exposed to the rat liver S9 preparation. Interestingly, the results also revealed that metabolic activation by rat liver S9 significantly increased their agonist/antagonist potential.

**TP258 Method Development for the Analysis of DDT, DDE, DDD in Cow Milk** A. Parra, Univ of Texas at El Paso, 330 Emerald Rise; B. Rocha, Univ of Texas at El Paso; W. Lee, Univ of Texas-El Paso. Organochlorine Pesticides (OCs) are classified as persistent organic pollutants (POPs). They have been found to bioaccumulate in organisms. These chemicals tend to attach to fatty tissues in animals and humans, remaining in them for long periods of time. OCs may be responsible for a vast number of health conditions including neurological disorders, endocrine disruption, reproductive dysfunctions, and possible linkage to cancer. OCs such as DDT, DDE and DDD are banned, however, they have still been detected in environmental samples worldwide. The main objective of this study is to investigate a new extraction method to determine the presence of DDT, DDE and DDD in milk using an innovated and environmental friendly technique, called Stir Bar Sorptive Extraction (SBSE). SBSE coupled with thermal desorption Gas Chromatography and Mass Spectroscopy will be applied in the analysis of OCs in samples. Different solvent system and additive effects were tested with SBSE process to determine OC recovery in milk samples. It was discovered that a 30% mixture of acetonitrile/ water and sodium hydroxide as an additive yielded the most OC recovery after extraction.

**TP259 Miniaturized Matrix Solid Phase Dispersion Extraction for the Analysis of Pesticides in *Chironomus dilutus*** Y. Ding, Southern Illinois Univ; A. Hartmann, Southern Illinois Univ, Fisheries and Illinois Aquaculture Center; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology. A miniaturized method using disposable glass Pasteur pipettes (5.75") was developed based on matrix solid phase dispersion extraction to measure seven pesticides and their degradation products in the aquatic invertebrate, *Chironomus dilutus*. The target pesticides included the organochlorine pesticide dichlorodiphenyltrichloroethane (*p,p'*-DDT), and its major degradation products, dichlorodiphenyldichloroethane (*p,p'*-DDD) and dichlorodiphenyldichloroethylene (*p,p'*-DDE); pyrethroid insecticides, permethrin, bifenthrin, and tefluthrin; and organophosphate pesticide, chlorpyrifos. The method compared Florisil and silica gel as dispersants for extraction and cleanup of biological samples. Analyses were performed by gas chromatography with micro-electron capture detection (GC- $\mu\text{ECD}$ ) with 4, 4'-dibromooctafluorobiphenyl and decachlorobiphenyl as spike surrogates. Silica gel (0.5 g) was selected as the dispersion absorbent to blend with midge larvae (0.05 g wet weight) and anhydrous sodium sulfate, while 4 ml ethyl acetate was used as the elution solvent. Mean recoveries ranged from 57.2 to 127.6% at spiked levels between 20 and 1000  $\mu\text{g kg}^{-1}$ , with relative standard deviations below 15% for most target analytes. The method detection limits ranged from 3.1  $\mu\text{g kg}^{-1}$  for *p,p'*-DDD to 19.6  $\mu\text{g kg}^{-1}$  for permethrin. The proposed method was used to measure *C. dilutus* residues of tefluthrin, chlorpyrifos, and *p,p'*-DDD in laboratory-exposed toxicity tests. The developed method was easier and less time consuming than the conventional extraction procedure and reduced the amount of sample, dispersant and solvent used.

**TP260 National Rivers and Streams Assessment Survey: Evaluating the Extent of Selected Human Prescription Pharmaceuticals in US Surface Waters** A.L. Batt, USEPA Office of Research and Development, National Exposure Research Laboratory; J. Lazorchack, L. Stahl, J. Wathen, USEPA. The Ecological Exposure Research Division (EERD) has prioritized a list of the most prescribed active pharmaceutical ingredients (API) in the US based on the potential of their wastewater residues to cause biological effects. A method using selective solid phase extraction and liquid chromatography in combination with tandem mass spectrometry was developed for the analysis of the top rated APIs from that process, which includes 48 APIs and 6 selected metabolites. The National Rivers and Streams Assessment (NRSA) is a statistical survey of flowing waters of the US designed to assess the condition of the Nation's rivers and streams. EERD from the Office of Research and Development (ORD) is working with the Office of Water (OW) on the current NRSA Survey, which consists of approximately 700 sites where fish were collected over the course of summer 2008 and 2009. A portion of these sample collection sites (180) have been identified as Urban Rivers, due to their proximity to urban areas. Surface water samples were collected from each of these Urban River sites and analyzed for the presence of over 50 human prescription pharmaceuticals. Several of the target analytes were detected, with concentrations ranging from low ng/L up to 500 µg/L. Results from this study will be presented and used by ORD and OW to assess the extent of contamination of these emerging contaminants in our nation's urban rivers.

**TP261 Occurrence and Environmental Distribution of Artificial Sweeteners in the Aquatic Ecosystems in Japan** T. Orishikida, H. Nakata, Univ of Kumamoto, Graduate School of Science and Technology; T. Hosono, Univ of Kumamoto, Priority Organization for Innovation and Excellence; M. Ono, T. Tokunaga, M. Kagabu, J. Shimada, Univ of Kumamoto, Graduate School of Science and Technology. In recent years, large amounts of low-calorie sweeteners have been used in a wide variety of soft drinks and foodstuffs in the world. These compounds are less degradable during wastewater treatment process, and detected in the aquatic environment in European countries. However, there are little data of the occurrence, concentrations and distribution of artificial sweeteners in Japan. In this study, we developed simultaneous analytical method of five artificial and two natural sweeteners in liquid samples and it was applied to wastewater treatment plant (WWTP), river water, and groundwater samples collected in Japan. As for the method development, the solid phase extraction (SPE) cartridge, Oasis HLB (500 mg, 6 cc), was used for the extraction of samples. When the eluate of SPE was applied to 40% acetonitrile/water (v/v), the recoveries of artificial and natural sweeteners were 92 and 87%, respectively. The artificial sweeteners, such as acesulfame K (AK) and sucralose (SUC), were detected in WWTP influents and effluents at the concentrations of > 1 µg/L. High concentrations of saccharin Na (SA) was also found in the influents of WWTPs, which suggests the large amounts of productions and usage of these compounds in Japan. AK, SUC and SA were detected in river water samples at the levels ranging from several tens to hundreds of ng/L. AK and SUC showed higher concentrations in river water samples at the stations proximal to downstream of WWTPs. AK and SA were also detected in groundwater samples, at the levels of

**TP262 Occurrence of Perfluorinated Compounds in the Ground and River Water of the Tama Region in Tokyo, Japan** T. Suzuki, Tokyo Metropolitan Institute of Public Health, Division of Water Quality; Y. Kosugi, M. Hosaka, K. Yaguchi, D. Nakae, A. Ogata, Tokyo Metropolitan Institute of Public Health; T. Nishimura, National Institute of Health Sciences. Perfluorinated compounds (PFCs) in the environment have been concerned by their persistence, bio-accumulation and toxicity. PFCs were detected in many kinds of samples from aquatic and atmospheric environment, food, human, and marine life. Perfluorooctane sulfonate (PFOS) and perfluorooctane sulfonyl fluoride were added to a list of persistent organic pollutants (POPs), by the Stockholm Convention in 2009. In this study, the monitoring of PFCs in the urban river water and groundwater at the Tama region in Tokyo, Japan was conducted. The analytes in this study were 10 perfluoro carboxylic acids (PFCAs, C5 – C14) and 4 perfluorosulfonates (PFCSS, C4 – C10). The river water samples of the Tama River, a typical urban river in the Tokyo area, were collected in 2008. The groundwater samples at the Tama region were collected from shallow and deep wells used as the drinking water from 2007 to 2010. The analysis was performed by a solid-phase extraction and a liquid chromatography-tandem mass spectrometry (LC/MS/

MS). PFCs in the river water samples obtained at the upper reaches ranged at the concentrations of 0.7 – 5.1 ng/L for PFCAs and 0.2 – 30.4 ng/L for PFCSS. Those obtained at the lower reaches of the river increased to 14 – 60 ng/L for PFCAs and 17 – 168 ng/L for PFCSS. The composition of PFCAs and PFCs were as follows: PFNA (43%) > PFOA (32%) > PFHxA (9%), and PFOS (77%) > PFHxS (18%) > PFBS (4%), respectively. The increase of the PFCs concentration in the river water was due to the inflow from sewage treatment plants (STPs) located by the river. The total inflow amount from the STPs ranged from 20 to 30 g/day. The higher concentrations of PFCs were detected in the groundwater samples than in the river water samples. The composition of PFCs in the groundwater samples (n=326) were as follows: PFOA (67%) > PFOS (57%) > PFHxS (55%) > PFNA (44%) > PFHpA, PFPeA, PFBS, PFHxA (35–42%) > the others (0–15%). PFCs in the groundwater samples ranged at the concentrations of 0.5 – 200 ng/L for PFCAs and 1.6 – 5800 ng/L for PFCSS. According to a cluster-analysis, PFOS and PFHxS were predominant in the groundwater in this region. The concentration of PFOS in the 16 wells exceeded the USEPA's provisional drinking water guideline value of 200 ng/L, whereas, the concentrations of PFOA in all wells were lower than the guideline value of 400 ng/L.

**TP263 Partitioning of a Suite of Chlorobenzenes to Aldrich Humic Acids and Primary Effluent Colloidal Organic Matter from a Sewage Treatment Plant** K. McPhedran, R. Seth, Univ of Windsor, Environmental Engineering; K.G. Drouillard, Univ of Windsor, Great Lakes Institute for Environmental Research. Municipal sewage treatment plants (STPs) can be a source of exposure to human and ecological receptors for many contaminants of concern through loadings via STP effluent discharges to receiving waters or land application of sludges. These loadings depend on the fate and transport of contaminants through the stages of the STP process. Submicron size or colloidal organic matter (COM) possess large surface areas that can facilitate binding of hydrophobic organic compounds (HOCs). Municipal sewage has a significant concentration of COM that can impact the HOC fate. HOC sorption to colloids has been well studied in natural environments; however, few studies have investigated their sorption to STP colloids. The objective of the current study is to improve understanding of HOC fate during the STP process and ensuing environmental loadings. In this study, the sorption of three chlorobenzenes (1,2,4,5-tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene) to COM in 1.5 µm filtrate of primary settling tank STP effluent was examined using the long-column sparging technique. Six spargers were used in parallel which allowed for concurrent replication of control and treatment spargers. Chlorobenzenes (CBs) are a diverse chemical suite with widely varying physico-chemical properties, presenting an ideal choice for better understanding of partitioning behaviour. Sorption of CBs to standard Aldrich humic acids (AHA) was conducted for comparison. Results show significant sorption of all three CBs to primary settling tank COM. The sorption behaviour was different as compared to AHA when normalized to the organic matter content. AHA sorption behaviour was similar to literature values and followed expected trends based upon physico-chemical properties of the three CBs. In contrast, no clear trends of sorption behaviour with effluent COM was observed. Implications of the results will be discussed.

**TP264 Persistent Organic Pollutants and Trace Elements Associated to Sedimentary Processes in the Colorado River Delta, Baja California, México** K. Lugo-Ibarra, Universidad Autonoma de Baja California, Instituto de Investigaciones Oceanologicas; L. Daessle Heuser, J. Macias-Zamora, N. Ramirez-Alvarez, Universidad Autonoma de Baja California. Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and trace elements (As, Cu and Pb) were studied in sediments cores from two distinctive modern channels of the Colorado River (CR) delta. Their abundance and temporal changes are associated with flood-flows of the CR across the Mexico-USA border. The CR channel is directly exposed to river flood-flows. The Hardy River (HR) is a local channel derived mainly from agricultural runoff, geothermal effluents and treated urban wastewater. Different headwater compositions and degrees of exposure to flood-flows appear to be the factors controlling the composition of persistent organic pollutants (POPs) and trace elements. Enrichment of OCPs (46 ng g<sup>-1</sup> dwt in HR and 4.37 ng g<sup>-1</sup> dwt in CR) occurred during, or a few years after flooding. PCB-138 (4.2 ng g<sup>-1</sup> dwt) is enriched in HR suggesting its origin in dielectric oils from the geothermal power plant. PCB-28 (2.1 ng g<sup>-1</sup> dwt) in CR maybe related with atmospheric input and/or re-deposition of upstream sediments. Only HR with 4, 4'-DDE (8.16 ng g<sup>-1</sup> dwt) and ΣDDT (8.34 ng g<sup>-1</sup> dwt)



exceed some of the international sediment quality guideline criteria. As, Cu and Pb suggest the most likely sources for these element enrichment especially As are the arsenate based pesticides used intensively in the area during the first half of the 20<sup>th</sup> century. Arsenic in the buried clay units of the HR has concentrations above the probable toxic effect level (PEL) for dwelling organisms, with maximum concentrations of 30 lg g<sup>-1</sup>.

**TP265 Photochemical Fate of Organic Ultraviolet Filter Chemicals in the Aquatic Environment** L.A. MacManus-Spencer, S. Bercovici, S. Suhag, A. Kracunas, Union College, Dept of Chemistry. One class of pharmaceuticals and personal care products of current environmental concern comprises the organic ultraviolet filter chemicals (UVFCs) used in sunscreen lotions, lip balms, and other personal care products to protect the skin from harmful UV radiation. In addition to indirect inputs (e.g., showering and washing clothes, leading to discharges to wastewater treatment plants), these chemicals also enter the aquatic environment by direct routes (e.g., swimming). Given the ever-increasing use of sunscreen products and their multiple routes to the environment, it is important to understand their environmental fate in order to make educated decisions about their regulation and remediation. In this study, the photochemical fates of two commonly used organic UVFCs, octyl dimethyl para-aminobenzoic acid (padimate O) and octyl methoxycinnamate (octinoxate), have been investigated. In previous studies, we have shown that octinoxate degrades readily by direct photolysis (half-life less than one hour), and we have identified several degradation products. In recent research, we have investigated the effects of sorption to suspended particles on the photolysis kinetics and product distribution of octinoxate. In addition, we have investigated the kinetics of padimate O photolysis under a variety of conditions. Padimate O degrades only slightly slower than octinoxate, with an estimated half-life of just over one hour, and is characterized by a direct photolysis quantum yield similar to that of octinoxate ( $\sim 10^{-3}$ ). Using liquid chromatography-mass spectrometry (LC-MS), several padimate O photodegradation products have been identified, including demethylation products and a methylation product. We have also obtained evidence of products formed by the reaction of two padimate O molecules, and some evidence points to the photochemical production of para-aminobenzoic acid (PABA), a previously used sunscreen chemical that is no longer approved by the FDA due to its ability to cause allergic reactions and its potential carcinogenicity. Finally, we have investigated the photolysis of mixtures of organic UVFCs in order to more accurately represent conditions both in the aquatic environment and in the sunscreen products themselves. For example, oxybenzone (another of the most commonly used organic UVFCs) acts to slow the photolysis of octinoxate, thought it does not appear to affect all photolytic pathways equally.

**TP266 Rapid Screening for Polyaromatic Hydrocarbons in Seafood Using LC-Fluorescence** J. Burgess, Waters Corp.; M. Benvenuti, K. Rosnack, Waters Corporation. Major oil spills such as the *Exxon Valdez* in 1989 and the recent Gulf of Mexico spill of April, 2010 have raised concerns over the quality of seafood harvested from these regions. Fish, crustaceans and mollusks may come into contact or ingest the oil thereby introducing potential health risks to consumers. PolyAromatic Hydrocarbons (PAHs) are an important subset of compounds present in oil that have been classified as priority pollutants. The USFDA has established levels of concern ranging from 0.035 mg/kg Benzo[a]pyrene in finfish to 2000 mg/kg combined phenanthrene and anthracene in oysters. To prevent consumption of contaminated seafood and minimize the impact on the seafood industry, a fast screening method is required to analyze these compounds of concern at the stated levels. Here we demonstrate that, following a simple extraction using a QuEChERS technique, an analysis of PAHs can be achieved in less than 4 minutes using the ACQUITY® H-class with fluorescence detection. Results show that dispersive sample preparation provides a fast and effective method for extracting PAHs from different seafood matrices. The method provides advantages over other sample preparation techniques as accurate results can be achieved with less sample preparation and in a shorter time. With sample preparation times reduced, a rapid chromatographic separation is critical to manage the samples, standards and QCs generated using this approach. The ACQUITY H-Class separation, which was achieved in less than 4 minutes, is able to address this demand. This solution allows laboratories to screen for PAHs in seafood, providing results in a timely manner.

**TP267 Sorption of Two Growth Promoters Used in Livestock to Midwestern Soils** A. Kreinberg, The Ohio State Univ, School of Earth

Sciences; Y. Chin, The Ohio State Univ, Dept of Geological Sciences. The environmental fate of synthetic and naturally produced hormones/growth promoters is not well understood. Tylosin is a macrolide antibiotic that is used both to treat bacterial infections in humans and more commonly as a growth promoter for swine production. As of 2000, over 90% of swine production facilities in the United States used antibiotics, of which tylosin was the most widely applied. Progesterone, an estrogenic steroid produced naturally by both genders, is also used (in conjunction with other steroids and steroid-like compounds) as a growth promoter in feedlot cattle. Both of these compounds are mostly metabolized by the target organism, but residual levels can enter the environment by waste products. Thus, sorption to soils by these compounds is a potentially important attenuation process. We conducted batch sorption experiments to measure the sorption of these two compounds to several different soils with different sorbent properties, such as soil organic matter content, cation exchange capacity, and soil pH. Our results show that tylosin sorption is linear to all soils with a soil organic matter partition coefficient ( $K_{OC}$ ) ranging from 446 to 1130 L/kg. Our  $K_{OC}$  values are consistent with values reported in the literature and are higher than the compound's octanol/water partition coefficient ( $K_{OW} = 316$ ). Tylosin has a  $pK_a$  of 7.7 and is positively charged at pH values less than its  $pK_a$ . We suspect that the higher than anticipated  $K_{OC}$  values reflect adsorption of the charged tylosin species to specific sites on the sorbent. Conversely progesterone sorption is non-linear and is best described by the Freundlich equation with slopes varying from 0.82 to 0.98. The degree of nonlinearity is strongly dependent upon the sorbent used. We are currently studying how both the sorbate and sorbent properties are influencing the sorptive behavior of our target compounds.

**TP268 Synthesis and Evaluation of Natural Product Halogenated Bipyrroles as Heme Oxygenase-1 Inhibitors** K. Foster, R. Smith, Univ of Mississippi, Dept of Medicinal Chemistry; D. Stec, Univ of Mississippi Medical Center, Dept of Physiology and Biophysics; J. Rimoldi, Univ of Mississippi, Professor of Medicinal Chemistry, Univ of Mississippi, Dept of Medicinal Chemistry. Naturally occurring halogenated bipyrroles have recently been discovered in seabird eggs bioaccumulating in trophic organisms. These compounds are suspected to be biogenic in nature from evidence of marine natural products persistent in the environment and widespread in the Pacific and Atlantic Ocean. These compounds exhibit chlorinated and brominated substitution patterns that closely resemble anthropogenic pollutants like the polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and polybrominated diphenyl ethers (PBDEs). In 2003, environmental health concerns surfaced regarding these naturally occurring bipyrrole compounds since several of these analogs were shown to induce the cytochrome P4501A (CYP1A) gene through activation of the AhR signaling pathway and also resulted in porphyrin accumulation in chicken embryo hepatocytes. Based on these studies, we have focused our efforts on examining the effects of halogenated bipyrroles to inhibit heme oxygenase (HO), the enzyme responsible for the catalytic oxidation of heme, which may account for the accumulation of porphyrins in hepatocyte models. Within the current study we have synthesized and characterized several natural product halogenated bipyrroles, and examined their effect on heme oxygenase-1, utilizing assays performed on liver lysates obtained from mice treated with the heme oxygenase-1 inducer, cobalt protoporphyrin. The ability of the halogenated bipyrroles to inhibit HO was evaluated by measuring the amount of bilirubin formed from the HO-catalyzed oxidation of hemin. Several of the analogs were found to be potent inhibitors of HO, and these results suggest that porphyrin accumulation from halogenated bipyrrole exposure may be mediated by the direct inhibition of heme oxygenase-1.

**TP269 Uptake Kinetics and Long-term Monitoring of Persistent Organic Pollutants Using SPMD and High Volume Water Sampler** G. Han, Korea Ocean Research and Development Institute, Oil & POPs Research Group; S. Hong, Korea Ocean Research & Development Institute, Oil and POPs research group, Korea Ocean Research & Development Institute; U. Yim, S. Ha, J. An, W. Shim, Korea Ocean Research and Development Institute. Passive sampling in environmental research is getting increasing attention as an alternative to expensive and more laborious active sampling. It can provide time-integrated samples for air and water. Uptake kinetics and characteristics of persistent organic pollutants (POPs) in seawater was investigated using passive and active water sampling. Semipermeable membrane devices (SPMDs) and high volume water sampler was deployed at a coastal station, Jangmok, Korea. Performance reference compounds

including PCB14, PCB50, PCB188, and  $\alpha$ -HCH-d6 were used to estimate the sampling rate of SPMD. Accumulation characteristics of POPs were monitored monthly basis by both sampling methods. In general, the POPs concentration in seawater tends to increase at high temperature season. Concentrations of POPs estimated by the SPMDs were comparable to those detected by active sampling. However, significant difference was observed in some volatile compounds such as HCHs, where active sampler accumulated relatively higher amount of the residues than SPMD. The isomer profiles of target compounds were similar between active and passive samples. The highest uptake rate of hydrophobic compounds by SPMDs was observed for analytes with log KOW between 5.0 and 6.5.

**TP270 Environmental Risk Assessment of Selected Human Pharmaceuticals in Urban Rivers in Japan** T. Nishimura, National Institute of Health Sciences, Division of Environmental Chemistry, National Institute of Health Sciences, Environmental Chemistry; A. Hirose, National Institute of Health Sciences, Risk Assessment, Biological Safety Research Center; T. Kawamoto, Hyogo Prefectural Institute of Public Health and Consumer Sciences Public Health Science Research Center, Life science division; M. Yano, Hyogo Prefectural Institute of Public Health and Consumer Sciences Public Health Science Research Center; Y. Kosugi, T. Suzuki, Tokyo Metropolitan Institute of Public Health, Water Quality. Recently, the environmental risk assessment (ERA) has been conducted in Japan. In EU and USA, the ERA of pharmaceuticals has been already introduced in order to develop a pharmaceutical with low potential environmental risk. The objective in this study was to investigate whether it is adjusted to apply the European Medicines Agency (EMA) guideline to Japanese situation by evaluating measured (MECs) and predicted (PECs) environmental concentrations of selected human pharmaceuticals in urban rivers receiving discharge from sewage treatment plants (STPs). This case studies were conducted at the urban river basins in Tokyo Metropolitan and Hyogo Prefecture in Japan. The MECs of the selected pharmaceuticals in water samples were measured by reverse-phase LC/MS/MS after solid-phase extraction. The PECs for aquatic environment of pharmaceuticals were calculated by according with Phase I of the EMA guideline. The PECs of the selected 101 pharmaceuticals in raw sewage of STPs were calculated from 0.10 to 300  $\mu$ g/L. 38 pharmaceuticals have been detected in raw sewage of six STPs. Their MECs ranged from 0.04 to 16.9  $\mu$ g/L, and all of them were lower than those PECs. As for the river basins, the PECs of 101 pharmaceuticals in the river water receiving discharge from STPs were calculated from 0.015 to 30  $\mu$ g/L. 41 pharmaceuticals have been detected in the river water. Their MECs ranged from 0.003 to 3.23  $\mu$ g/L and were in the same range of the PECs. In the river water at the sampling site, where approximately half of the river water consists from drainage of STPs, however, the MECs of epinastine (antiallergic drug), candesartan (drug for high blood pressure), and lorazepam (psychotropic drug) were higher than their PECs. Epinastine is mainly excreted unchanged in human. Candesartan, principle active metabolite of candesartan cilexetil in human, is excreted unchanged in urine and feces. Lorazepam is mainly excreted as the glucuronide. The removal rates of these active gradients at six STPs ranged from 0 to 30%. The pharmaceuticals, which have maximum dose lower than several dozen mg/kg/day, high excretion ration in human, and low removal rate at STPs, could be underestimated in urban river in Japan at the dilution factor (DF) = 10 in Phase I of the EMA guideline. However, it was prevent when the DF of real field condition at the sampling sites in the river were applied.

**TP271 Optimizing Scientific and Social Attributes of Pharmaceutical Take Back Programs to Improve Public and Environmental Health** K.L. Stoddard, Univ of North Texas, Institute of Applied Sciences, Univ of North Texas, Research assistant; D. Huggett, Univ of North Texas, Institute of Applied Sciences. Scientific data indicate that pharmaceuticals are present in water, sediment, and aquatic biota here in the US and in many other countries. Research continues to provide evidence that these contaminants may cause adverse environmental effects with the most studied response being feminization of fish associated with oral contraceptives. Excess pharmaceuticals also pose a threat to public health due to the increased risks of accidental poisoning and prescription medicine abuse. The two main routes by which pharmaceuticals enter the environment are improper disposal of excess or unwanted pharmaceuticals and post-consumer excretion. Pharmaceutical take-back programs (TBP) have emerged as a promising method for addressing these public and environmental health concerns; however, to date oversights of scientific and social elements of TBPs have limited their

full potential to address these concerns. A semi-annual TBP called Denton Drug Disposal Day (D4) has been developed in Denton, Texas in response to these oversights. The objectives of D4 include providing a safe pharmaceutical disposal method, determining the public and environmental health benefits of a TBP, and using survey analysis and Geographic Information Systems (GIS) to optimize TBPs. Prior to the first D4 event, a phone survey of Denton County residents was conducted to gather baseline information on disposal practices, public risk perception, and identify barriers to TBP participation. Data from this survey were analyzed using logistic and linear regression models to identify factors that significantly influence an individual's willingness to participate and willingness to pay for TBPs. Additionally, data from D4 participant surveys were also analyzed to determine behavioral trends and the geographic distribution of D4 participants. Knowledge gained from these analyses will be applied to future D4 events to improve event participation and effectiveness. Environmental health benefits of D4 events are currently being assessed through bimonthly monitoring of a target pharmaceutical in local wastewater effluent using LC-MS/MS. Public benefits of TBPs will be determined by comparing poison data before and after D4 events to determine if human drug poisonings have been reduced. These latter analyses will provide critical data necessary to determine if D4 events can improve both public and environmental health.

**WP001 Quantification of Manufactured TiO<sub>2</sub> Nanoparticles in UK Natural Water Systems** G. Raza, J. Lead, Univ of Birmingham, School of Geography Earth and Environmental Sciences. Titanium dioxide (TiO<sub>2</sub>) nanoparticles are excessively used in a wide range of consumer products, mounting a big concern on ultimate fate of these materials in aquatic environment. The current research focuses on behaviour of some of the smallest TiO<sub>2</sub> nanoparticles (5-30 nm in diameter), in a range of UK river waters having different chemistry. Different phase distribution of such nanoparticles has been synthesized in School of Geography, Earth and Environmental Sciences Univ of Birmingham and Natural History Museum London. River water samples (with different water chemistry) to be analysed are collected from different parts of United Kingdom with help of Centre of Ecology and Hydrology. All samples are being analysed for any initial amount of TiO<sub>2</sub> present. In next phase effect of manufactured nanoparticles (NP's) on overall water chemistry will be analysed by spiking known quantity of NP's. The parameters which need to be addressed are pH, zeta potential, electrical conductivity, total organic carbon (TOC), dissolved oxygen (DO) and different cations & anions within natural waters. The ultimate fate of TiO<sub>2</sub> nanoparticles as a function of above mentioned parameters will be studied in depth.

**WP002 Assessment and Source Modeling of Bioavailable Contaminants in Gulf of Mexico Coastal Waters Before, During and After the Deepwater Horizon Oil Spill** S.E. Allan, Oregon State Univ, Environmental and Molecular Toxicology, Oregon State Univ, Graduate Researcher; B. Smith, Oregon State Univ; K.A. Anderson, Oregon State Univ, Environmental & Molecular Toxicology. Following the fatal explosion of the Deepwater Horizon oil rig, lipid free tubing, low-density polyethylene passive sampling devices (PSDs) were deployed in coastal marine waters of four states in the Gulf of Mexico; Louisiana, Mississippi, Alabama and Florida. A pre-impact baseline was obtained prior to shoreline oiling in May, 2010 followed by 13 months of sampling during and post shoreline oiling. Samples were analyzed for unsubstituted polycyclic aromatic hydrocarbons (PAHs), methylated PAHs and oxygenated PAHs, and screened for over 1200 chemicals of concern using Deconvolution Reporting Software (Agilent Technologies). PSDs sequester and concentrate the freely dissolved, and therefore bioavailable, fraction of hydrophobic organic contaminants, providing a time integrated measure of their concentration in the environment. This research is a follow-up to 5 months of data that was presented at the SETAC NA 2010 meeting. Since then, sampling continued through June, 2011 and the samples have been analyzed for a wide range of chemicals associated with crude oil and its breakdown products. The highest concentrations of PAHs in coastal marine waters were observed at the site in Louisiana during June and July; demonstrating a more than 40 fold increase over pre-impact levels. The other sites showed maximum concentrations up to 10 times above baseline levels and a west-to-east temporal trend in the timing of peak concentrations. The bioavailable concentration of PAHs in coastal waters was not significantly elevated above pre-oiling baselines at any of the sites by May, 2011. Other organic contaminants assessed in this study demonstrated temporal changes that suggest an association with crude oil contamination events and analysis of PAH degradation products provides insight into the fate of certain chemicals of concern in the environment. Chemical data was modeled to elucidate changes in chemical profiles associated with unique source inputs. Among other models, principal component analysis discerned three distinct groups of samples based on chemical profile. Pre-oiling samples from all four sites were separated from two "oiling" groups, where the Louisiana site had a distinct profile from the three eastern sites. Samples from spring, 2011, had chemical profiles that associated them with the pre-oiling group, demonstrating an attenuation of the input from the unique Deepwater Horizon spill source.

**WP003 Desorption and Bioavailability of Organic Contaminants in Biosolids** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering, Univ of California, Dept of Civil and Environmental Engineering; H. Kim, Univ of California, Civil and Environmental Engineering. Biosolids contain variety of toxic chemicals, including legacy and emerging organic compounds and trace metals. Toxic chemicals in biosolid amended surface soils are subject to wash off by stormwater runoff, which transports toxic chemicals into adjacent creeks, rivers, and estuaries. Toxic chemicals desorbed from biosolids can be also taken up by plants and worms and eventually may be transferred to higher trophic level wildlife such as birds and mammals. It is important to know desorption rates of toxic chemicals in biosolids to predict their wash off rates and bioavailability.

We conducted batch tests to investigate desorption of PAHs in biosolids with an aid of ethyl vinyl acetate (EVA) as adsorbent added to accelerate desorption. Vials (50 mL) containing biosolid (1 g), EVA (1 g), and water were tumbled for 0, 3, 6, 9, 12, 24, 48, 72, and 96 hours. EVA collected at each time point was extracted with hexane to quantify the mass of desorbed PAHs. PAH desorption rates negatively correlated with their hydrophobicity (Kow). Acenaphthene and fluorene reached equilibrium within 48 hours but 4-ring PAHs such as fluoranthene, pyrene, benz[a]anthracene, and chrysens didn't reach equilibrium during the study period. Desorption of 5- and 6-ring PAHs was negligible compared to smaller PAHs. Additional tests with longer mixing time (e.g., weeks or months) will be conducted to obtain more accurate desorption rates and equilibrium time of high molecular weight PAHs. Batch test samples will be also analyzed to verify whether the results of the present study can be used to predict other hydrophobic organic contaminants having similar Kow values.

**WP004 Direct and Indirect Photochemistry of Sulfapyridine and Sulfamethoxazole in the Aquatic Environment** J.K. Challis, Univ of Winnipeg, Richardson College for the Environment; J.C. Carlson, Univ of Winnipeg, Richardson College for the Environment, Univ of Manitoba, Dept of Environment and Geography; K.J. Friesen, Univ of Winnipeg, Richardson College for the Environment; M.L. Hanson, Univ of Manitoba, Dept of Environment and Geography; C.S. Wong, Univ of Winnipeg, Richardson College for the Environment, Richardson College for the Environment, Environmental Studies Program and Dept of Chemistry. The averaged direct photolytic quantum yield of the sulfonamide antibiotic, sulfapyridine, was characterized over a 290-300 nm wavelength range as a function of pH, to assess the ability of sulfapyridine to undergo direct photolysis, an important contributor to its degradation in the aquatic environment. Time-based direct photochemical rate constants and quantum yields were determined using a merry-go-round photochemical reactor for this chemical in singly deprotonated (pH 5) and doubly deprotonated (pH 11) states, as well as at an environmentally relevant pH of 7. The second pKa of sulfapyridine was measured via spectrophotometric titration to be 8.58±0.02, a result previously unreported. The photochemical reactivity of sulfapyridine increased with pH, despite a reduction in its molar absorption coefficient. The quantum yields at pH 5, 7, and 11 were 0.002 ± 0.001, 0.003 ± 0.001 and 0.02 ± 0.01 respectively. This increase in quantum yield with pH has been observed with other sulfonamides, but not sulfapyridine. The relatively large quantum yields at environmentally relevant pH values and the significant light absorption (molar absorption coefficient > 100) at near-UV wavelengths suggest that sulfapyridine will be quickly photodegraded in surface waters. Significant indirect photolysis was suggested by field experiments in sewage lagoons with significant amounts of dissolved organic matter.

**WP005 Levels of PBDEs and Hexabromobiphenyl (BB 153) in Surface Water and Bottom Sediment of Diep River, Cape Town, South Africa** A.P. Daso, Cape Peninsula Univ of Technology, Cape Town, South Africa., Environmental And Occupational Studies, Cape Peninsula Univ of Technology, Environmental And Occupational Studies; O.S. Fatoki, Cape Peninsula Univ of Technology, Cape Town, South Africa., Dept of Chemistry; J.P. Odendaal, O.O. Olujimi, Cape Peninsula Univ of Technology, Cape Town, South Africa., Dept of Environmental and Occupational Studies.; H.K. Okoro, B.O. Opeolu, Cape Peninsula Univ of Technology, Cape Town, South Africa., Dept of Chemistry. Polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) are important brominated flame retardants that have attracted global attention in the past two decades. Unfortunately, there is still paucity of environmental data with respect to these widely studied contaminants in Africa and in most developing countries around the world, hence, concerns on how to bridge this knowledge gap necessitate this study. In this study, river water (n=51) and bottom sediment (n=54) samples were analysed for the concentrations of the commonly investigated PBDE congeners (BDE 28, 47, 100, 99, 154, 153, 183 and 209) as well as BB 153. Samples were collected bi-monthly between May 2010 and March 2011 from three sampling locations. These locations correspond to the upstream, point of discharge and downstream regions of a wastewater treatment plant, which discharges its effluents into the river. The routine analysis of these samples was carried out using GC-ECD technique while the structural elucidation and confirmation of target compounds were done using gas chromatography (time-of-flight, TOF) mass spectrometry. The overall mean values of all the eight PBDE congeners in the sediment



and river water samples are as follows: 0.69, 0.34, 0.45 ng/g (dw) and 1869.90, 4051.77, 116.07 ng/L for upstream, point of discharge and downstream samples, respectively. Surprisingly, BB 153 was detected at a relatively high level than expected with overall mean values of 103.83, 284.33, 116.93 ng/L and 0.12, 0.09, 0.19 ng/g for river water and sediment samples, respectively. BDE 47 was the most dominant congener in the river water contributing more than 95% to the total PBDE concentrations, except for the samples collected at the downstream region where it ranked second next to BDE 153 contributing 19.0% to the total PBDE concentrations. In sediment samples, BDE 209, 47, 183 and 99 were the dominant congeners. Due to the lower concentrations of BDE 209 found at the upstream regions with correspondingly higher values further downstream, it thus implied that discharges from the WWTP might be a significant source of BDE 209 into the Diep River. The trend observed in this study revealed that there could be other notable sources of PBDEs into the river other than from the WWTP.

**WP006 Levels, Removal Trend and Health Risk of Metals (As, Cd, Pb, Hg, Ni, Cr, Co, Zn, Cu) from Wastewater Effluents in Cape Town, South Africa** O.O. Olanrewaju, Cape Peninsula Univ of Technology, Environmental And Occupational Studies, Univ of Agriculture, Environmental Management & Technology; O.S. Fatoki, Cape Peninsula Univ of Technology, Cape Town, South Africa., Dept of Chemistry; J.P. Odendaal, Cape Peninsula Univ of Technology, Cape Town, South Africa., Dept of Environmental and Occupational Studies.; A.P. Daso, Cape Peninsula Univ of Technology, Cape Town, South Africa., Environmental And Occupational Studies, Cape Peninsula Univ of Technology, Environmental And Occupational Studies; H.K. Okoro, B.O. Opeolu, Cape Peninsula Univ of Technology, Cape Town, South Africa., Dept of Chemistry. This study focused on one year monitoring campaign of occurrence and removal of endocrine disruptive metals (EDMs) from selected wastewater treatment plants (WWTPs) in Stellenbosch and Cape Town. Water samples were collected from the WWTPs from January 2010 to December 2010 on quarterly basis and metals concentration determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) after open beaker. A total of 432 water samples consisting of raw, primary effluent, secondary effluent and final effluents were collected. The general abundance distribution pattern for metals is  $Zn > Cu > Pb > Cr > Ni > As > Co > Cd > Hg$ . The removal efficiency ranged from 1.5% for Hg at Zandvliet WWTP plant during winter to 98.27% for Cu at Athlone WWTP treatment plant during summer. The final effluent concentration for most of the metals were within South Africa water quality guidelines while As, Hg, Cd and Pb concentration were higher than maximum limits set by Canadian council of ministers of the environment. Potsdam WWTP plant proved to be more effective at heavy metals removal as compared to the other five treatment plants investigated in this study. The effluent metal concentration over time could pose health risk if used for vegetable watering.

**WP007 Occurrence of Bisphenol A in Paper Currencies from Several Countries and Implications for Dermal Exposures** C. Liao, K. Kannan, New York State Dept of Health, Wadsworth Center. Bisphenol A (BPA) is one of the highest production volume chemicals and has been widely used in industrial and commercial products. In this study, paper currencies ( $n = 156$ ) collected from 21 countries from March 2010 to January 2011 were analyzed for the presence of BPA. Three circle punches (19 mm diameter) were taken from the lower left corner, middle, and upper right corner of each paper currency. Samples were cut into small pieces, spiked with  $^{13}C_{12}$ -BPA as an internal standard, and then extracted with methanol 3 times and subjected to high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. BPA was found in all currencies at concentrations ranging from 0.001 to 82.7 (mean 5.21; median 1.13)  $\mu g/g$ . BPA concentrations in samples taken from the middle portion of the currencies were higher than those taken from the peripheral portions. Transfer of BPA from thermal receipt paper to currencies was also examined by placing currencies in contact with thermal receipt papers for 24 h in a wallet. Concentrations of BPA increased by 2-3 orders of magnitude after 24 h of contact with thermal receipt papers (mean  $\pm$  SD,  $18.0 \pm 11.1 \mu g/g$  after contact versus  $0.068 \pm 0.077 \mu g/g$  before contact; paired  $t$ -test,  $p < 0.01$ ). This result clearly suggests the transfer of BPA from receipt paper to currency bills and that thermal receipt paper is a major source of BPA found in paper currency bills. Estimation of daily intake of BPA through dermal absorption from handling of paper currencies showed that exposures from currencies is a minor pathway, although high levels of BPA were measured.

Since the paper currency is frequently handled in daily life, exposure doses of BPA can be elevated under occupational settings.

**WP008 Perfluorinated Compounds in Human Blood, Water, Edible Freshwater Fish and Seafood in China: Daily Intake and Regional Differences in Human Exposures** T. Zhang, Nankai Univ, China, and Wadsworth Center, USA, Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, Nankai Univ; H. Sun, Y. Lin, L. Wang, X. Zhang, Y. Liu, MOE Key Laboratory of Pollution Processes and Environmental Criteria, Nankai Univ; X. Geng, Waters Technologies (Shanghai) Ltd; F. Li, L. Zhao, MOE Key Laboratory of Pollution Processes and Environmental Criteria, Nankai Univ; K. Kannan, Wadsworth Center, New York State Dept of Health and Dept of Environmental Health Sciences, School of Public Health, State Univ of New York at Albany. Despite the growing public interest in perfluorinated compounds (PFCs), very few studies have reported the sources and pathways of human exposure to these compounds in China. In this study, concentrations of 10 PFCs were measured in human blood, water (tap water and surface water), freshwater fish and seafood samples collected from China. Based on the data, we calculated daily intakes of PFCs, regional differences in human exposures, and potential risks associated with ingestion of PFCs from diet, drinking water, and indoor dust for the Chinese population. Perfluorooctane sulfonate (PFOS) was the most predominant PFC found with a mean concentration of 12.5 ng/mL in human blood from Tianjin, and 0.92 ng/g wet wt in freshwater fish and seafood; perfluorooctanoic acid (PFOA) was the major PFC found in drinking water at a concentration range of 0.10 to 0.92 ng/L. The estimated daily intake of PFOS and PFOA via fish and seafood consumption (EDfish&seafood) ranged from 0.10 to 2.51, and 0.13 to 0.38 ng/kg bw/d, respectively, for different age groups (i.e., toddlers, adolescents and children, and adults) from selected locations (i.e., Tianjin, Nanchang, Wuhan, Shenyang). The EDfish&seafood of PFCs decreased ( $p < 0.05$ ) with age. The estimated daily intake of PFOS and PFOA via drinking water consumption (EDIdrinking water) ranged from 0.001 to 0.009, and 0.002 to 0.027 ng/kg bw/d, respectively. Comparison of EDfish&seafood, and EDIdrinking water values with the modeled total dietary intake (TDI) of PFCs by adults from Tianjin, Nanchang, Wuhan, and Shenyang showed that contributions of fish and seafood to TDI of PFOS varied depending on the location. Fish and seafood accounted for 7%, 24%, 80%, and 84% of PFOS intake in Nanchang, Shenyang, Wuhan, and Tianjin, respectively, suggesting regional difference in human exposure to PFOS. Drinking water was a minor source of PFOS ( $< 1\%$ ) exposure in adults from all the study locations.

**WP009 Relationship Between Soil Sorption Coefficient (K<sub>OC</sub>) and Octanol/Water Partition Coefficient (K<sub>OW</sub>) for Methylsiloxanes** S. Xu, Dow Corning Corporation, Health and Environmental Sciences; G. Kozerski, Dow Corning Corporation, Health & Environmental Sciences. In environmental modeling, organic carbon normalized soil/water sorption coefficients ( $K_{OC}$ ) of nonionic organic compounds by natural organic matter are often estimated from their octanol/water partition coefficients ( $K_{OW}$ ) using an empirical single-parameter linear relationship. When this approach was used for cyclic methylsiloxanes (cVMS) such as octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), the measured log  $K_{OC}$  values for cVMS were found to be much smaller than those estimated based on the empirical model optimized using data for non-siloxane organic compounds as the training set. In order to better understand the relationship between  $K_{OC}$  and  $K_{OW}$ , the measured room temperature values of log  $K_{OC}$  and log  $K_{OW}$  from literature were analyzed in this study for nonionic (neutral) organic compounds, including volatile methylsiloxanes (VMS). It was found that the aforementioned discrepancy for siloxanes arose from two factors. First, relative to wet n-octanol, wet organic carbon is a more cohesive matrix, stronger H-bonding donor, and more effective at inducing dipoles in polarizable molecules. Secondly, relative to the benchmark non-organosilicon compounds for which the correlation between  $K_{OC}$  and  $K_{OW}$  was derived, VMS have larger size, strong H-bonding basicity and low polarizability that signify the difference in solvation properties between organic carbon and octanol. Consequently, VMS follow the same scientific principles as other nonionic organic compounds in environmental partitioning, but may not obey the simplified single-parameter relationships.

**WP010 Spiders and Spider Silk as Bioindicators for PCB-contaminated Sediments** J. Dahle, Clemson Univ, Dept of Chemistry, 342 Computer Court; D. Delach, Clemson Univ, Environmental Toxicology; C.M. Lee,

Clemson Univ, Environmental. Engineering and Earth Sciences Dept; V. Dang, Clemson Univ, Environmental Engineering and Earth Sciences. Currently the USEPA and others employ expensive and time-intensive testing methods to quantify the concentrations of polychlorinated biphenyls (PCBs) in sediments of hazardous waste sites. In this study, spiders and spider webs from the Sangamo Weston/Twelvemile Creek/Lake Hartwell Superfund Site (SW/12MC/LH), SC, USA, were collected and tested to determine their accuracy as PCB bioindicators. Spiders and webs from both lotic and lentic systems were collected and their accuracy as concentration markers were compared and contrasted. Concentrations in spiders have recently been recorded as high as 1800 ppb and in webs 361 ppb. Low concentrations in spider webs versus the bodies of the spiders may indicate that webbing is not a highly efficient excretion pathway for PCBs. The derived relationship between PCB levels in sediment and the bodies of spiders and their webs will be presented. This study also seeks to quantify chiral preferences of PCB accumulation in spider webbing as an excretion pathway; this characteristic could be useful in further studies that investigate how the incorporation of PCBs and other contaminants into spider webbing affects properties of the silk. Thus far, spider webbing has been found to select chirally for most congeners, such as PCB 95 which exhibits an average enantiomeric fraction of 0.144. Previous research exists which indicates that spiders are an accurate model of PCB concentrations in the SW/12MC/LH area. The collection of spiders and spider webs is fast, inexpensive, and easy; these samples are also easy to extract and analyze, with extraction requiring less solvent (as little as 15 mL) and sample mass (as little as 10 g) than sediment testing. These benefits coupled with studies such as this one showing the accuracy of spiders and their webs as PCB concentration models could help lead to the development of less expensive, "greener" contaminant testing methods for contaminated sediments.

**WP011 The Fate of Perchlorate in a Man-made Reflecting Pond Following a Fireworks Display in Albany, New York, USA** Q. Wu, School of Public Health, State Univ of New York at Albany, Environmental Health Sciences, Wadsworth Center, New York State Dept of Health, New York State Dept of Health, Wadsworth Center; J. Oldi, Wadsworth Center and Dept of Environmental Health Sciences, SUNY at Albany; K. Kannan, New York State Dept of Health, Wadsworth Center. Perchlorate is a widespread contaminant in aquatic environments. Despite this, aquatic environmental fate of perchlorate released from fireworks displays is not well known. In this study, we examined the fate of perchlorate in man-made reflecting ponds, from 2008 to 2010, following three fireworks displays in Albany, New York. Immediately after the fireworks display, perchlorate concentrations in pond waters increased significantly, with concentrations 30 to 1480 times higher than the baseline values. Perchlorate concentration in pond water increased from 0.11 µg/L to up to 519 µg/L, following the fireworks display in 2008. Perchlorate concentrations in pond water decreased at a first-order kinetic degradation rate, with a mean  $k_{obs}$  value of 0.026 d<sup>-1</sup> and an average half-life of 29 d. The rate of perchlorate deposition into water bodies following fireworks displays was estimated to range from 670 to 2620 g/ha. We also estimated the perchlorate ingestion rate by the inhalation of aerosols of pond water by people frequent near the pond. The estimated daily intake of perchlorate through the ingestion of aerosols was 32% (226 ng/kg bw), 13% (92 ng/kg bw), and 6% (42 ng/kg bw) of the US Environmental Protection Agency's reference dose for infants, children, and adults, respectively.

**WP012 Toxicity of Manufactured CuO Nanoparticles in *Caenorhabditis elegans*** Y. Zhang; B. Zhang, East Carolina Univ, Dept of Biology, East Carolina Univ, Biology, East Carolina Univ, Dept of Biology. Although manufactured metal oxide nanoparticles (NPs) have been widely used in various applications, limited information is available on the potential impacts of NPs on the human health and on the ecological systems. In this study, we use *Caenorhabditis elegans* (*C. elegans*) as an animal mode to assay the toxicological effects of CuO nanoparticles. The *C. elegans* L4 larvae was dosed with different concentrations (0, 0.001, 0.01 and 0.05 mg/mL) of CuO NP for three days. The growth and reproduction effects were investigated. In molecular level, we also dosed *C. elegans* L4 larvae for 24 hours to test the expression of egg-laying and oxidative stress related genes. Results showed CuO NP causes growth retardation and brood-size reduction in *C. elegans*. The gene expression of some egg-laying or stress related genes, such as *acr-8*, *egl-9*, *egl-46*, *old-1* were also affected by CuO NP.

**WP013 What Do We Know About What Is in Personal Care Products?** R. Dodson, Silent Spring Institute; M. Nishioka, Battelle Memorial Institute; L. Standley, L. Perovich, J. Brody, R. Rudel, Silent Spring Institute. Despite concerns about endocrine disruption and other health effects from chemicals in household and personal care products, ingredient labeling and safety information is limited, making it difficult to evaluate products as exposure sources or make informed exposure reduction decisions. To help fill this data gap, we analyzed personal care, cleaning, and other household products for a range of endocrine disrupting compounds (EDCs) and chemicals associated with asthma. We also evaluated whether package labels can be used to identify products without these chemicals. We tested 213 commercial products composited into 85 samples and representing 50 product types, including cosmetics, personal care products, cleaners, sunscreens, and vinyl shower curtain and pillow protector. Analytes included parabens, phthalates, bisphenol A (BPA), antimicrobials, ethanolamines, alkylphenols, fragrances, glycol ethers, cyclosiloxanes and ultra-violet filters. We detected 55 compounds, indicating a wide range of exposures from common products. Vinyl products contained >10% bis(2-ethylhexyl) phthalate (DEHP) and could be an important source of DEHP in homes. In other products, the highest concentrations and numbers of detects were in perfume, air fresheners, dryer sheets, and sunscreens. Some products marketed as "green" did not contain the well-known EDC phthalates but contained other less-studied phthalates that are also EDCs, suggesting a substitution. Common products contain complex mixtures of EDCs and chemicals associated with asthma that are also found in environments relevant to human and ecological risk. Many detected chemicals were not listed on product labels, but some, including synthetic fragrances, BPA, and active ingredients, appear to be avoided using general purchasing criteria. Findings have implications for human and ecological risk assessment, since certain chemicals tend to co-occur and product composition varies within a product type.

**WP014 Generating Screening-level Exposure Assessments of Personal Care Products in China at Multiple Spatial Scales** C.M. Holmes, R. Vamshi, D. Mao, Waterborne Environmental, Inc.; J. Hodges, O. Price, Unilever. The development and application of models to predict in-river concentrations of ingredients used in personal care products are important to the environmental risk assessment process. While suitable models exist in the US and Europe, there are currently no available models to estimate exposure of personal care products in China. To this end, work was performed to create a GIS-based system that develops exposure estimates used to predict the emission of personal care products into freshwater ecosystems. Currently, many of the available inputs for the generation of environmental concentrations to be used in the exposure assessment are at a very coarse spatial scale, in some cases only a single value for the entire country is available. In this study, environmental concentrations in surface water were estimated at the province and county-levels (ca. 2,900 counties in China) based on both user supplied product/ingredient information, as well as other geographically-linked socio-economic and environmental information from official census and other data sources. Product information such as category (e.g., hair, skin, etc.), composition (e.g., ingredient fraction) and "take off" values (GDP threshold under which the product would not likely be purchased) were used to distribute total tons of individual ingredients based on product sales data in China. These data were combined with economic information, population density (including urban and rural separation), water use, disposal mechanism (e.g., STP, septic, direct discharge to river, etc.), and location-specific dilution factors to estimate ingredient-level concentrations in surface water at the county level. Results show that estimated concentrations vary considerably across the country when using more refined spatial data, and that economic information ("take off" values and population GDP) can have a significant influence on the resulting ingredient emission distributions. The method presented incorporates the inherent spatial variability of the model inputs so that patterns can be identified and used in the risk assessment. In other words, the ability to identify areas where existing combinations of model inputs may yield greater exposure estimates, i.e., the identification of realistic "worst case" scenarios. In addition, an understanding of where these "worst case" scenarios fit within the overall China-wide distribution is achieved.

**WP015 Influence of Land Use on Ecological Risk Assessment of Municipal Wastewater Treatment Plant Discharges** K. Kapo, Montani Run, LLC; S. Dyer, The Procter & Gamble Company, Central Product Safety, The Procter & Gamble Company, Miami Valley Innovation Center.

Ecological risk of consumer product ingredients associated with municipal wastewater treatment facility discharges may depend upon surrounding land use characteristics. A statistical comparison of biological condition (fish and invertebrate communities) between field monitoring sites (Ohio EPA) upstream and downstream of municipal wastewater treatment facility discharge points throughout the state of Ohio was conducted to evaluate potential impacts of chemicals and materials associated with municipal effluent. Land use-dependent relationships of upstream-downstream trends were delineated by comparing results between facilities located in areas of urban and rural land use, respectively (National Land Cover Dataset classifications). Comparisons of modeled effluent toxicity (consumer product ingredients and pharmaceuticals, respectively), other water chemistry parameters, habitat quality, and other landscape factors compiled from available data resources were also conducted between upstream and downstream sites. This study expanded upon a previous study (1996-1999 data) of major rivers ("RF1"), using updated data (2000-2008), additional environmental variables, and the National Hydrography Dataset stream network allowing for the inclusion of smaller tributaries. Evidence of a stronger influence of effluent in urban areas indicates that land use is a critical factor to consider in effectively characterizing the risk of effluent to receiving water ecosystems. The influence of urban vs. rural analysis on understanding the potential risks associated with domestic use of down-the-drain chemicals, such as those in consumer products will be highlighted. Understanding the importance of land use is imperative in subsequent experimental approaches designed to obtain quantitative causal relationships of domestically used chemicals.

**WP016 IFRA Environmental Standards: Risk and Hazard Assessment Update for 2011** A. Lapczynski, Research Institute for Fragrance Materials, Inc., Research Institute for Fragrance Materials, Inc., Environmental Specialist; D. Salvito, Research Institute for Fragrance Materials, Inc., Dept of Environmental Science; M. Vey, IFRA. The International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. The development of this program was reported previously at SETAC (Seville, 2010). To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the developing IFRA Environmental Standards, reported here are the results of these additional tests (for both risk and hazard assessments). These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

**WP017 Aquatic Ecological Assessment for Shampoos: Sensitivity of the USETox Model to Parameter Uncertainty** J.K. Saxe, M.L. Fleming, EcoSafety Sciences. The French Environment and Energy Management Agency (ADEME) is overseeing a new requirement for a comparative evaluation of the environmental performance of certain consumer products, including shampoos, to be provided to consumers. The USETox model was selected for this purpose. The aquatic environmental assessment in USETox is quantified as a single value, a characterization factor (CF). The quality of the model results and sensitivity of the model to three critical factors are presented. First, USETox requires a significant number of input parameters, many of which are not widely available for the range of ingredients used in shampoos. Specifically, the biodegradation rate is important in the model but adequate empirical data are often lacking for this parameter. Sensitivity of the model result to the use of empirical data vs. BIOWIN model results is examined. Second, surfactants are the most abundant non-water ingredient in shampoos, yet the environmental fate segment of the USETox model relies on a substance's octanol-water partition coefficient (Kow) – a parameter that is not meaningful for surfactants – to predict environmental partitioning. Two approaches for addressing the lack of a meaningful Kow for surfactants are compared. Third, aquatic toxicity is quantified using a

distribution approach rather than selecting a single study as the point of departure for determining a predicted no effects concentration. This approach requires more toxicity data than are available for many shampoo ingredients, and requires that all quality studies conducted for an ingredient be evaluated and incorporated into the CF determination. The use of modeled versus empirical data, as well as the influence of study selection versus rejection on the data distribution (and hence on the model results), are presented. The result of this evaluation shows that the model results are most sensitive to uncertainty around the effects factor, which can span orders of magnitude, compared to uncertainty around biodegradability and octanol-water partition coefficient for surfactants. This suggests that clear guidance around selection and use of effects data, which are applied consistently across ingredients, is critical to achieving quality results in aquatic assessments for shampoos in USETox.

**WP018 Toxicity of Decamethylcyclopentasiloxane (D5) to Aquatic and Terrestrial Environments** A. Fairbrother, Exponent, EcoSciences, Exponent, Inc., EcoSciences; A. Burton, Univ of Michigan, School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology (ENTOX). Decamethylcyclopentasiloxane (D5) is undergoing regulatory review in Canada and Europe to ascertain its persistence, bioaccumulation, and inherent toxicity. In this paper, we focus on the question of ecotoxicity, specifically toxicity of D5 to pelagic (water column), sediment, and soil organisms. We specifically investigated the exposure concentrations in the tests in relation to the solubilities of D5 in those media. We concluded that below the solubility limit of D5 in water (17 mg/L), no adverse effects of D5 have been observed in any of the acute or chronic laboratory studies with fish, *daphnia*, or algae. Because the concentration of D5 in environmental media cannot exceed its solubility, it follows that there currently is no evidence to support a finding that D5 can cause immediate or long-term harmful effect to water-column biota in the environment. For soil organisms, D5 shows only minor effects to plants and invertebrates, with no-observed effect concentrations (NOEC = 77 mg/kg-dw barley and 377 mg/kg-dw springtails) several orders of magnitude higher than D5 residues measured in the environment (0.006–0.221 mg/kg-dw). The lowest NOECs for sediment organisms (62 mg/kg-dw *Hyalella*; 70 mg/kg-dw *Chironomus*) are ten times as high as the highest value measured in sediment at wastewater treatment plant outfalls (5.8 mg/kg-dw). Critical body burdens for benthic invertebrates were derived by multiplying lipid-normalized NOECs by the D5 biota-sediment accumulation factors [BSAF] from field-collected invertebrates and comparing those to measured tissue concentrations from field-collected organisms. Similar results were seen; field organisms are not able to accumulate toxic amounts of D5. Therefore, we conclude that D5 is not toxic to aquatic or terrestrial organisms at environmentally-realistic concentrations.

**WP019 Environmental Assessment of D5 – Fate and Exposure** C.E. Cowan-Ellsberry, CE2 Consulting, LLC, Central Product Safety, CE2 Consulting; D. Mackay, Trent Univ; B. Price, The Procter & Gamble Company. D5, cyclopentasiloxane-decamethyl, is one of several siloxanes that are used in a variety of personal care products, including hair care, skin care, cosmetics and antiperspirants and deodorants. Environment and Health Canada conducted a PBT categorization and screening assessment of the siloxanes, including D5, in 2008. They concluded that D5 meets the criteria for persistence and bioaccumulation and that D5 is entering the environment in a quantity that may have a harmful effect on the environment. Therefore, D5 should be listed on the Toxic Substances list and management actions taken to control D5's release to the environment, including potentially virtual elimination. The Silicones Environmental Health and Safety Council of North America recognized shortcomings in the evaluations that had been performed and requested that a Board of Review be constituted to conduct a de novo review of the nature and extent of the risk posed by D5 to the environment. As part of this process, we evaluated the D5 fate and estimated the exposure in various environmental media for Canada. D5 is a very unusual chemical in part due to its large size and molecular volume, low water solubility and high vapor pressure. As a result D5 does not partition among water, organic carbon, octanol and biological fluids like traditional non-polar substances resulting in very limited solubility in water, soil and sediment. Once released into the environment, a majority of the D5 will partition to air where it undergoes hydrolysis. A small amount will remain



in water and sediment. The primary pathway to the aquatic and sediment environments is through consumer product release to sewers, therefore, the fate and resulting exposure to these media from sewage plant effluent were estimated. Comparison of these estimated exposures to recent monitoring data for Canada showed that these estimated exposures were conservative and reasonable. These data also show that the current exposures are well below the maximum solubility in the media and well below any toxic level of concern.

**WP020 Using a Novel Sediment Exposure to Determine the Effects of Triclosan on Marine Benthic Communities** K.T. Ho, USEPA, Atlantic Ecology Division; L. Portis, USEPA; M. Perron, Brown Univ, School of Engineering; M. Pelletier, National Health and Environmental Effects Research Laboratory, USEPA, Dept: ORD NHEERL AED; M. Cantwell, D.R. Katz, USEPA, Atlantic Ecology Division; A. Chariton, CSIRO; S. Simpson, CSIRO Land and Water, Centre for Environmental Contaminants Research; D. Proestou, USEPA, Atlantic Ecology Division; R.M. Burgess, USEPA, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, USEPA, USEPA; J. Baguley, Univ of Nevada, Dept of Biology. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol), is an emerging contaminant commonly used as an antimicrobial compound in many personal care products such as softsoap, detergent, toothpaste, mouthwash, and is infused in many consumer products, such as kitchen utensils, toys, bedding, socks, and trash bags. Triclosan enters estuarine environments primarily via wastewater treatment plant effluent and has been found in sediment cores dating back to the mid 1960s. The objective of our research was to determine the effect of triclosan on intact estuarine benthic communities. We adapted a novel exposure method which brings intact sediment cores into the laboratory and then exposes the benthic community by addition of toxicant spiked sediments to the core surface. Treatments included: 1) field control – no added sediment, 2) laboratory control – clean sediment added, 3) low triclosan concentration (~20 mg/kg dry), and 4) high (~200 mg/kg dry) triclosan concentration. Macro- and meiobenthic communities were analyzed for differences after a two week exposure to either high or low concentrations of triclosan spiked sediments. Results from the triclosan sediment additions indicated a loss in total abundance and number of meiofaunal and macrofaunal taxa communities at the high triclosan concentration compared to the control. This high exposure concentration is similar to LC50s for amphipods and mysids exposed in the laboratory. We also noted a difference in the field control relative to the laboratory control, most likely due to a smothering effect of the added sediment. Differences in benthic communities were not noted between the low triclosan and laboratory control treatments. These data suggest that at elevated concentrations, triclosan can disrupt benthic communities while also indicating the exposure system does not completely emulate exposures in the field.

**WP021 Determination of Cadmium Bioavailability of Winter Barley Through the Analysis of Guttation Water Droplets** D. Schenke, Julius Kühn-Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection; T. Strumpf, Julius Kühn-Institute; J. Krueger, Technische Universität Berlin. Guttation is known over hundred years, but this phenomenon was less considered in the system of soil/plant/atmosphere. The term guttation defines the exudation of water droplets through specific secretory organs at the edges of plant leaves in vascular plants. Guttation can be observed at high relative humidity when plants close their stomata and the transpiration suction is ceased in the order of 3 MPa. The smaller root pressure (0.3 MPa) caused by osmosis pushes the xylem-water droplets through the hydathodes (Schopfer u. Brennecke, 2006). In the guttation droplets mineral nutrients (Ivanoff, 1963), several amino acids (Pilot et al., 2004) and also active substances of plant protection products which may be applied as seed coating (Girolami et al., 2009, Schenke et al., 2010) were detected. This exposure by pesticide-containing guttation liquid may be relevant for water collecting bees and plant-settled non-target arthropods. Cadmium is transported with the transpiration stream up to the apical leaves in plants (Strumpf et al., 2009). In autumn 2010 winter barley (*cv. Naomi*) were sown in two slightly loamy soils with different cadmium content at the field station of JKI in Berlin. After emergence of winter barley coleoptiles guttation droplets were taken from the edges of leaves and analyzed. The concentration of cadmium in guttation droplets from barley at the contaminated soil (site A) exceeded clearly the concentration measured at the control site (B) with normal cadmium content. The cadmium concentration in guttation droplets in the early growth stages of barley (BBCH-code 10

– 11) were compared with cadmium content in soil measured with different methods (Tab.). The cadmium concentration in guttation water was found to be clearly lower than the content in the ammonium nitrate fraction which is the standard for assessment of metal bioavailability in Germany (BBod-SchV, 1999). Table: Cadmium in soil and guttation water droplets Method Conc. Site A Site B Soil/aqua regia digestion mg kg<sup>-1</sup> 18.50 0.491 Soil/ammonium nitrate extraction 0.187 0.003 Soil/calcium chloride extraction 0.029 0.002 Guttation water (barley BBCH 10-11) mg L<sup>-1</sup> 0.012 ± 0.005 0.0008 ± 0.0001

**WP022 Trace Element Contamination Around the E-Waste Recycling Site at Agbogbloshie, Accra City, Ghana** M. Otsuka, Ehime Univ, Center for Marine Environmental Studies; T. Itai, Ehime Univ, Center for Marine Environmental Studies (CMES); K.A. Asante, Ehime Univ, Center for Marine Environmental Studies (CMES), Ehime Univ, Center for Marine Environmental Studies; M. Muto, Center for Marine Environmental Studies (CMES), Ehime Univ; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies. E-waste, obsolete electrical and electronic material, has become a subject of growing environmental concern in developing countries due to the large volume of illegal import from developed nations. Many toxic substances including BFRs, PCBs and heavy metals contained in e-waste are released into the environment during disposal and recycling. Furthermore, inappropriate thermal treatment and combustion processes widely employed by recyclers in developing countries to retrieve valuable metals have been known to generate unintentional but highly toxic chemicals such as PCDD/Fs, PBDD/Fs and mixed halogenated homologues. The lack of data is most glaring with regard to the e-waste recycling site in Asian and African developing countries, where the pollution has often been overlooked. In this study, we investigated level and speciation of trace metals around the largest e-waste recycling site, Agbogbloshie market in Accra city, Ghana. Concentrations of 15 elements in soil/ash mixture collected around Agbogbloshie markets were measured by handheld XRF analyzer. Accuracy of XRF measurement was preliminarily evaluated by measuring 19 certified geological materials distributed by Geological Society of Japan. In most of the cases, accuracy was within an order of magnitude. Concentrations of Cu (50 to 22000 mg/kg), Zn (200 to 160000 mg/kg), Pb (100 – 14000 mg/kg), and Sn (< 50 to 1000 mg/kg) were extremely high in residual ash derived from wire combustion, and Br (20 – 1500 mg/kg), As (< 50 to 1100 mg/kg), and Hg (< 20 to 150 mg/kg) were also at moderately high levels. Concentrations of these metals were positively correlated with darkness of soil color suggesting that these levels were simply controlled by dilution of residual ash by uncontaminated soil. Hence, it can be presumed that these metals were from e-waste products, and released into the surrounding environment through open burning. X-ray absorption fine structure measurements revealed that majority of Br was possibly in the inorganic salt rather than organic contaminants. As the Br concentration is the highest in black ash, the source of Br might be anthropogenic compounds, such as BFRs. Predominance of inorganic Br in soil/ash mixture might be attributed to the conversion of organic Br by thermal decomposition. Formation of dioxin like compounds is probably high in this area, and hence further comprehensive monitoring is needed to assess the health risk for the e-waste recycling workers.

**WP023 Toxic Metal Contamination of Soil in Four Major Indian Cities** P. Chakraborty, Savannah State Univ, Dept of Natural Science; K. Sajwan, Savannah State Univ, Natural Science. India being a tropical country, organic matter has a very high decomposition rate. With the rapid industrialization and urbanization, the anthropogenic wastes from heavy industries, electronic waste recycling units and ship breaking sites are found to be the key factors contributing toxic metal contamination of soil in major cities of India. Nevertheless, toxic metals accumulate in the soil and present a serious long-term hazard and are a source of danger to the health of the people. Hence present study was designed to assess Pb, Cu, Zn, Co, Ni, As, Cd, Hg and Cr in the soil samples from four major cities of India. Surface soil samples (0-20 cm) were collected from 15-20 locations based on urban rural and background transect from each city viz., New Delhi located in the north, Kolkata in the east, Mumbai in the west and Chennai in the southern part of India. Sampling was conducted during December 2006 through June 2009. Recoveries were nearly quantitative for all the metals studied. Comparison of the average metal concentration levels with world averages indicates an elevated value for Pb for certain locations. The applicability of this method was crosschecked with AAS and the results were in good agreement. The overall range of concentration in mg/kg were 30-390 for Cr,

167-4370 for Mn, 4-100 for Co, 13-300 for Ni, 10-1930 for Cu, 0.3-50 for As, BDL-15 for Cd, 1-80 for Hg and 5-2340 for Pb. The data reveals that soils from the sites close to the electronic waste dismantling sites in New Delhi and ship breaking yard in Mumbai are significantly contaminated, showing higher levels of these toxic metals from those urban sites than normal distribution in the background sites. The level of toxic metal contamination in Kolkata and Chennai were lower compared to New Delhi and Mumbai.

**WP024 Trace Metals in Biosolids and Biosolid Amended Soils of Farm Land** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering, Univ of California, Dept of Civil and Environmental Engineering; H. Kim, Univ of California, Civil and Environmental Engineering. Large fraction of sludge from municipal wastewater treatment plants are being converted to biosolids to use as organic fertilizer. Most biosolids have been applied to farm lands to improve soil productivity and stimulate plant growth. Biosolids also contain very high levels of variety of toxic chemicals, including trace metals. This study investigated impacts of biosolid amendment on surface soil trace metal contamination. Surface soils were collected from farms in Solano County, California. These farms had received biosolids previously with different time interval (years). Biosolid analysis showed that concentrations of some trace metals such as copper, zinc, molybdenum, tin, and antimony were significantly higher in biosolids than in background surface soils. These metals in biosolid amended surface soils generally are up to 80-fold enriched. Among these metals, molybdenum, tin, and antimony can be used to indicators for biosolid application. Surface soils amended with biosolids more frequently had higher levels of metals, indicating biosolid application increases metal concentrations. Among measured metals, antimony and molybdenum in all biosolid amended soil exceed soil screening guideline values set to protect human health. Soil core data and plant bioaccumulation data will be added to the presentation.

**WP025 PCBs, PBDEs, and OCPs in *Seriola lalandi* from the Subtropical Convergence Zone of the North Pacific Gyre** S. Harwani, California Environmental Protection Agency, Dept of Toxic Substances Control, Environmental Chemistry Laboratory, Dept of Toxic Substances Control, California Environmental Protection Agency; J. Park, California Environmental Protection Agency, Dept of Toxic Substances Control, California Environmental Protection Agency, Research Scientist; T.Y. Garcia, Environmental Chemistry Laboratory, Dept of Toxic Substances Control, California Environmental Protection Agency; M. Gassel, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency; A. Neal, Jean-Michel Cousteau's Ocean Futures Society; B. La Belle, M. Petreas, Environmental Chemistry Laboratory, Dept of Toxic Substances Control, California Environmental Protection Agency. Over the last two decades the North Pacific Ocean has garnered much attention due to the noticeable amounts of mega-, macro- and micro-debris that has been found floating within it and being ingested by biota. The key portion of this marine debris is from plastics which do not generally biodegrade, but instead break down to smaller micro-plastic fragments. The ingestion of macro- and micro-plastics and the ensuing physical harm to biota has been a vital concern. More recent studies indicate that new and emerging persistent organic pollutants (POPs) have a high affinity for plastics and may adsorb onto them and get ingested by biota. Consequently it is even more important to further understand the dynamic role and impact that marine debris may have on the marine environment. In August of 2009, Project Kaisei launched its first expedition to the North Pacific Ocean with these objectives. During the early part of the expedition, 19 *Seriola lalandi* (Yellowtail amberjacks) fish samples were collected from the Subtropical Convergence Zone of the North Pacific Gyre. A simple and efficient methodology for the preparation, extraction, cleanup, and analysis of the fish sample extracts for polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs) was developed. In these remote ocean-surface fish, legacy OCPs were found to be at high concentrations ( $1480 \pm 241$  ng/g lipid weight), while total PCBs and PBDE levels were an order of magnitude lower on average with concentrations of  $359 \pm 56.1$  ng/g lipid weight and  $204 \pm 9.1$  ng/g lipid weight, respectively. The results from these studies warrant further investigation of various exposure pathways, including plastics, to these fish, and marine biota, in general. In addition to these studies, work focusing on PCB and PBDE metabolites in these fish will be pursued. "The views expressed herein are those of the authors and do

not necessarily reflect those of Dept of Toxic Substances Control, California Environmental Protection Agency."

**WP026 Accumulation Features of Halogenated Phenolic Contaminants in the Blood of Pinnipeds Along the Japanese Coastal Waters** K. Nomiyama, C. Kanbara, H. Mizukawa, A. Eguchi, M. Ochiai, Ehime Univ, Center for Marine Environmental Studies (CMES); T. Isobe, Ehime Univ, Senior Research Fellow Center, Ehime Univ, Senior Research Fellow; T.K. Yamada, National Museum of Nature and Science, Tokyo, Dept of Zoology; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies. Information on accumulation features of polychlorinated biphenyl metabolites (OH-PCBs) and hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in the blood of marine mammals is limited. In this study, we determined the residue levels and patterns of PCBs (mono- to deca-, 62 isomers), PBDEs (mono- to deca-, 14 isomers), OH-PCBs (tri- to octa- 52 isomers), OH-PBDEs (tri- to octa-, 28 isomers) in the blood of pinnipeds [Northern sea lions (*Eumetopias jubatus*: male = 6, female = 4), Northern fur seals (*Callorhinus ursinus*: male = 3, female = 7), ribbon seals (*Phoca fasciata*: male = 1, female = 1) and spotted seals (*Phoca largha*: male = 6, female = 2)] along the Japanese coastal waters. Penta- through hepta-chlorinated PCB congeners were the dominant homolog groups in all species. The dominant PCB isomers found in all pinnipeds were CB153 and CB138. In contrast, specific differences in the distribution of dominant OH-PCB isomers and homologues were found among the species. The mean concentrations of  $\Sigma$ OH-PCBs were  $180 \text{ pg g}^{-1}$  in spotted seals,  $160 \text{ pg g}^{-1}$  in ribbon seals,  $52 \text{ pg g}^{-1}$  in Northern fur seals and  $6.8 \text{ pg g}^{-1}$  in Northern sea lions. The predominant OH-PCB isomers were para-OH-PCBs such as 4OH-CB107 and 4OH-CB187 in Northern sea lions. 4OH-CB107, 4OH-CB146, 4OH-CB162 and 4OH-CB187 were predominant in ribbon seals. 4OH-CB107 and 4OH-CB162 were predominant in ringed seals. In Northern fur seals, 4'OH-CB101/4'OH-CB120, 4OH-CB107, 4OH-CB162 and 4OH-CB97 were predominant. The differences in concentrations and profiles of OH-PCBs in the pinnipeds may suggest species-specific diets and metabolic capability. Characteristic differences found in the homolog patterns of OH-PCBs in pinnipeds suggest the need for further studies on the differences in exposure profiles, metabolic capacities and toxic effects. Of the 28 OH-PBDE isomers monitored, only two isomers (2'OH-BDE68 and 6OH-BDE47) could be consistently measured above the limit of quantification in all pinnipeds. The two abundant isomers accounted for about 41–99% (6OH-BDE47) and 1–48% (2'OH-BDE68) of the sum of all identified OH-PBDEs. These compounds have been reported as natural products present in red algae and cyanobacteria. It is probable that the many 6OH-BDE47 and 2'OH-BDE68 in cetaceans are not the metabolites of anthropogenic PBDEs.

**WP027 Characterization and Quantification of PCB Metabolites in Riparian Spiders** D. Delach, Clemson Univ, Environmental Toxicology; C.M. Lee, Clemson Univ, Environmental Engineering and Earth Sciences Dept; D. Walters, USGS, Fort Collins Science Center, USGS, Ecologist. Many organisms can transform PCBs into various metabolites, such as hydroxylated PCBs (OH-PCBs). A variety of organisms ranging from polar bears to pinnipeds to fish have been examined; however, invertebrate metabolic capacity has only begun to be studied. It has been postulated that OH-PCBs can mimic edysone hormone that stimulates exoskeleton shedding, but there has been little further investigation. Spiders have the important role of mediating trophic transfer from aquatic to terrestrial ecosystems, and may have the capacity to metabolize contaminants. Data from GC-MS-MS analysis provides qualitative evidence that the riparian orb weavers *Tetragnathidae* spiders have body burdens as high as 2400 ng/g, and may have the capacity to metabolize the contaminants. Hydroxylated PCB congeners with varying levels of chlorination are present, including di-, tri-, tetra-, and penta-chlorinated congeners. In an effort to determine the enzymatic detoxification pathway a suite of biomarkers was enlisted. Assays to assess activity of the most likely agents of detoxification were performed – namely, for cytochrome P450 isoforms 1A (EROD) and 2B (PROD), and for glutathione enzymes (GST assays). They were performed with spider tissue obtained along the contamination gradient. Preliminary results have indicated that CYP2B does not have a role in the detoxification process, unlike higher order organisms such as trout and birds.

**WP028 The Immunologic Effects Aged Oil Sands-derived Naphthenic Acids on Rainbow Trout Co-exposed to Pathogen Associated Molecular**

**Patterns** G.Z. MacDonald, Canadian Rivers Institute, Univ of Prince Edward Island; N.S. Hogan, K.L. Thorpe, L.J. Phalen, M.R. Heuvel, Canadian Rivers Institute, Univ of Prince Edward Island, Biology. Fishes in oil sands affected ponds and lakes have demonstrated an increase in disease prevalence which has been correlated with exposure to constituents of waters influenced by oil sands. Naphthenic acids are the major organic constituent in these waters raising questions regarding the potential of naphthenic acids to cause immune suppression in fishes. To investigate this potential, rainbow trout were injected with naphthenic acids extracted from 17-year-old oil sands tailings pond water using an acid precipitation method. Spectrophotometric analysis on this extract showed results consistent with the spectra of extracted naphthenic acids. Fish were injected with 0, 10, or 100 mg/kg dose of extracted naphthenic acids and sampled after either 5 or 21 days. The fish from the 21-day exposure were co-exposed to an injection of formalin inactivated *Aeromonas salmonicida* to induce an immune response. To assess the immune response, relevant immune parts including blood, spleen, and head kidney were removed from the fish at the end of the exposure and the constituent leukocytes, T lymphocytes, B lymphocytes, thrombocytes and myeloid cells were counted and typed via flow cytometry analysis using fluorescent antibodies specific for those cell types. In the 5-day exposure, there was a general depression in whole white blood cell populations and spleen thrombocyte populations with a significant depression at the 100 mg/kg dose. In the 21-day exposure white blood cell populations in both low and high doses showed some recovery from effects observed in the 5-day exposure, and spleen thrombocyte populations showed a general increase with dose with a significant increase at the 100 mg/kg dose. In the 21-day exposure, many cell types consistently showed a significant Dose x *A.s.* interaction at 100 mg/kg. Control fish cell numbers were consistently stimulated by *A.s.* injection while naphthenic acid injected fish usually showed a minor depression in numbers as compared to fish coexposed to *A.s.* From these results, it appears that naphthenic acids stimulate the immune response rather than suppress it. Overall, naphthenic acids are likely acting via a generally toxic mechanism in the short term, an immunostimulatory mechanism in the long term, and not via a specifically immunotoxic mechanism in either case.

**WP029 Speciation of Vanadium in Coke and Oil Sands Process Water by HPLC-ICP-MS** M. Jensen-Fontaine, Univ of Alberta, Chemistry; C. Le, Univ of Alberta, Dept of Laboratory Medicine & Pathology; X. Lu, Univ of Alberta, Laboratory Medicine & Pathology; P. Pourrezai, Univ of Alberta, Civil and Environmental Engineering; M. Gamal El-Din, Univ of Alberta, Dept of Civil and Environmental Engineering. The Athabasca Oil Sands, found in Northern Alberta, Canada, produce over a million barrels of oil a day. The mining and upgrading process creates several by-products including petroleum coke and oil sands process water (OSPW). Most of the coke is stockpiled for use in future land reclamation projects. Some coke is slurried with OSPW to settling basins. Chemicals in the OSPW can sorb onto the coke or leach from the coke into the OSPW during the slurrying. Leaching of metals from coke during storage and after its use in land reclamation projects is also an environmental concern. Speciation and oxidation state affect a metal's toxicity, leaching potential and bioavailability. Of the six different oxidation states of vanadium, the common ones found in nature are V(III), V(IV), and V(V). The most toxic oxidation state is V(V). There are high levels of vanadium (640 ppm) in the Athabasca Oil Sands, mostly V(IV), that can potentially oxidize to V(V). To evaluate the severity of potential environmental contamination due to vanadium leaching and oxidation, we optimized an HPLC-ICP-MS method for vanadium speciation. The technique was applied to coke and OSPW samples. The limit of detection for both V(IV) and V(V) was below 1 µg/L. V(IV) rapidly oxidized in OSPW so a sample stability method was developed. Complexation with EDTA preserved the sample speciation and concentration for the duration of the study (56 days). The half-life of V(IV) in OSPW was determined by spiking OSPW with different concentrations of V(IV) and observing the conversion from V(IV) to V(V). In previous work, we had determined the speciation and concentration of vanadium in the supernatant when coke and OSPW were mixed for 12 hours. We therefore investigated the initial leaching profile of vanadium from the coke into OSPW. The supernatant was speciated at regular intervals during the first six hours of mixing. Over 60% of the vanadium leached in the first two hours. V(V) was the major species present in the supernatant as was the case with the 12 hour mixing tests. Future work will involve performing a bioaccumulation study of vanadium in *Hyalella azteca* by speciating the invertebrates and the water.

**WP030 Are Oil Sands Sediments Toxic to Northern Pike (*Esox lucius*)?** D. Turcotte, Environment Canada, Environment Canada; J.C. Raine, Toxicology Centre; L.K. Romanowski, V. Tumber, Environment Canada; J.L. Parrott, Environment Canada, Environment Canada, National Water Research Institute, Environment Canada. Northern pike (*Esox lucius*) are a commercially important fish species native to the northern hemisphere. In Alberta (Canada), these fish inhabit the Athabasca River, which flows through the Athabasca oil sands, and are exposed to natural sources of bitumen eroding from the McMurray formation. There is currently no information available to assess the early development of pike exposed to the bitumen present in the water of the Athabasca River. Pike are not easily cultured in a laboratory environment and no methods have been developed to assess the toxicity of oil sands to this fish species. Thus the current study describes the design and implementation of a daily-renewal bioassay that assesses the potential effects of sediments from the Athabasca oil sands area to the early stages of pike development. Eggs were collected and fertilized with milt from spawning wild pike captured from Lake Diefenbaker, SK. The fertilized eggs were exposed to treatments containing different concentrations of sediments from the oil sands area, reference sediments or strictly culture water until complete yolk absorption of control fish, approximately 15 days post-hatch. Brine shrimp were fed to the pike embryos daily at the initiation of exogenous feeding and continued to the conclusion the experiment. Developing fish were examined for morphological deformities, survival, hatching success, and changes in weight and length between treatments. Preliminary results suggest that the Northern pike is less sensitive than walleye and fathead minnow to the toxicity of oil sands sediments.

**WP031 Characterization of Athabasca Oil Sands Process Water by Comprehensive Two-dimensional Liquid Chromatography** M.S. Ross, Y. Wang, Univ of Alberta, Dept of Laboratory Medicine & Pathology; S.J. Rowland, Univ of Plymouth, School of Geography, Earth and Environmental Sciences; J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine & Pathology. The toxicity of oil sands process water (OSPW) has been mainly attributed to a complex mixture of large (C<sub>8</sub>-C<sub>22</sub>) aliphatic and alicyclic carboxylic acids, called naphthenic acids (NAs; C<sub>n</sub>H<sub>2n-2</sub>O<sub>2</sub>). However, a number of NA-like compounds containing heteroatoms (i.e., N, S, O), aromatic groups, or multiple carboxylic acid moieties are also reported to be present in OSPW. Partial identification of OSPW components often relies on ultra high resolution mass spectrometry (MS), but even by this method the 'identification' of compounds is limited to chemical formula, with little information about chemical structure. Two-dimensional (2D) gas chromatography-MS techniques have allowed identification of numerous NA, including in OSPW, and shown promise for characterizing other OSPW components, but usually has the disadvantage of low resolution mass spectrometry and often also requires a derivatization step. The goal of the present work was to further characterize NA and NA-like components of OSPW using a novel comprehensive two-dimensional (2D) HPLC with high resolution or tandem mass spectrometry detection. Using a combination of a ThermoFisher Hypercarb column in the first dimension and a C18 monolithic column in the second dimension, NAs were fully resolved from their oxidized degradation products and other isobaric acid-extractable compounds. Structure-retention relationships were studied by utilizing a series of "model" naphthenic acid compounds. By doing so, the retention behavior was calibrated, allowing for the prediction of compound structure based on retention time in the two-dimensional system. In the first dimension, NAs could be separated from their oxidized products, while a combination of first and second dimension separation was needed to separate NAs from aromatic and heteroatom containing compounds. Furthermore, the improved chromatographic resolution over one-dimensional systems allowed for the collection of tandem mass spectra of individual naphthenic acid components, aiding in compound identification. The 2D HPLC method was further applied to investigate the unique compound fingerprints in commercial naphthenic acids, authentic OSPW, and environmental samples from the Athabasca River and surrounding area.

**WP032 Synchrotron Technology Used to Identify the Presence of Multiple Elements within Naphthenic Acids Extracts Derived from Process-affected Waters** N. Toor, Level Science Inc., Environmental Science; C.H. Ryan, Y. Hu, Canadian Light Source Inc., J. Headley, Environment Canada, Water Science and Technology Directorate. Naphthenic acids (NAs) and related components derived from oil sands process-affected waters (OSPW) have not been completely characterized using conventional



analytical techniques. As a novel alternative, synchrotron technology can provide valuable information on the elemental composition of OSPW NAs. However, this method has the added benefit of being able to identify different elemental oxidation states, local molecular geometries, and chemical forms based on the absorption spectra generated during analysis. To date, both sulfur and boron components have been identified and speciated in these complex NAs mixtures. Additionally, the Soft X-Ray Microcharacterization Beamline (SXRMB) equipped with a Princeton Gamma-Tech (PGT) multi-element X-ray detector was used to complete a broad spectrum analysis of concentrated NAs extracts. Elements such as Cu, Ni, Mn, Cr, Fe, V, K, Cl, and Ca were identified in select extracts derived from regional process-affected and natural water sources. Differences in these components and their oxidation states could inevitably be used as potential markers for delineating anthropogenic impacts. This research highlights the potential for synchrotron technology, used alone or in combination with other analytical methods, to enhance the current understanding of the complex acid-extractable organic fraction of OSPW, which in the past has simply been referred to as naphthenic acids in the classical sense.

**WP033 A Comparison of Reproductive Performance of Yellow Perch Held in Experimental Ponds Containing Oil Sands Affected Waters Over a 13-year Period** M.R. Vandenheuvel, Canadian Rivers Institute, Univ of Prince Edward Island; N.S. Hogan, Canadian Rivers Institute, Univ of Prince Edward Island, Dept of Biology; S. Roloson, Canadian Rivers Institute, Univ of Prince Edward Island; G. Van Der Kraak, Univ of Guelph, Dept of Integrative Biology. The oil sands of Northern Alberta produces large volumes of tailings and process affected waters that must be safely incorporated into the reclaimed landscape. Unextracted materials in exposed oil sands or lean oil sands incorporated into reclaimed area may also chemically influence water bodies. In this study we describe the results of stocking experiments with yellow perch held in two experimental ponds over a 13-year period. Demonstration Pond contained oil sands fine tailings capped with natural surface water whereas South Bison Pond contained unprocessed oil sands material. Yellow perch were captured from a nearby reservoir, Mildred Lake, and placed in the two experimental systems. Two natural lakes, one at the edge of the Athabasca oil sands deposit, and one removed from the formation were also used for comparison purposes. The initial stocking experiments conducted from 1995 to 1997 showed that both male and female yellow perch had increased gonad size in the experimental ponds as compared to Mildred Lake and that gonad size and fecundity fell within, or was greater than that observed in the natural reference lakes. The only difference in sex steroid hormones were significant reductions in both testosterone and 11-ketotestosterone in males in the Demonstration Pond and South Bison. Yellow perch successfully reproduced in the ponds during the 1995 to 1997 period. In a repeat study conducted in 2009, male yellow perch in the Demonstration Pond had the lowest gonad size while the South Bison Pond had the highest gonad size. This was paralleled by the Demonstration Pond males having significantly lower testosterone and 11-ketotestosterone than all other populations while South Bison Pond perch steroid concentration were higher than all other populations. Female yellow perch gonad size was not significantly different than the other populations but plasma estradiol and testosterone was significantly lower than the other sites. The reproductive results are consistent with an observed increase in oil sands-related contaminants in the Demonstration Pond as the tailings consolidate over time, releasing material in the water column. Conversely, organics in the South Bison Pond decreased after the area was reclaimed in 1996. Results also suggest a more severe effect of unknown oil sands constituents in male perch than in female perch.

**WP034 Evaluating the Effects of Naphthenic Acid Fractions on *Pimephales promelas*** A.E. Bauer, Univ of Waterloo, Dept of Biology; R.A. Frank, Environment Canada, National Water Research Institute; L. Bragg, Univ of Waterloo, Dept of Biology; M. Kohli, M. Hewitt, Environment Canada, National Water Research Institute; D.G. Dixon, Univ of Waterloo; M.R. Servos, Univ of Waterloo, Dept of Biology and Canadian Water Network, Univ of Waterloo, Dept of Biology. Naphthenic Acids (NAs) are a complex group of alkyl-substituted acyclic, monocyclic and polycyclic carboxylic acids, with surfactant properties that are released from oil sands during bitumen extraction. In fact, they have been deemed the most toxic component of oil sands process material and process water (OSPW). Studies have indicated that an increase in NA concentration within reclamation pond water increases the potential for detrimental reproductive effects, as

well as increased larval mortality and pathologies in fish. High molecular weight NAs are degraded more slowly than low molecular weight NAs resulting in a higher proportion of high molecular weight NAs in aged OSPW. Furthermore, studies have identified a decrease in toxicity associated with aged OSPW and degraded NAs. Few studies have evaluated the toxicity associated with a fractionated extract of oil sands NAs. This study assesses the difference in toxicity associated with different molecular weight fractions of an oil sands NA extract on the fathead minnow (*Pimephales promelas*). The NA extract was fractionated by distillation, allowing for the collection of fractions by molecular weight. Each fraction was then tested at multiple concentrations with an acute fathead minnow 7-d larval bioassay to determine 7-d LC50s and IC25s. These endpoints were then compared such that a lack in overlap of 95% confidence limits indicated a significant difference. Initial results suggest a potential for low molecular weight NAs to be more toxic to fathead minnows than high molecular weight NAs.

**WP035 The Effects of Oil Sands Naphthenic Acids on the Growth and Physiology of *C. reinhardtii*** K.L. Goff, Univ of Saskatchewan, Toxicology; J.V. Headley, Environment Canada, AEPRD; J.R. Lawrence, Water Science and Technology Directorate, Environment Canada, Water Science and Technology Directorate; T. Ellis, Canadian Light Source; K.E. Wilson, Univ of Saskatchewan, Biology. Studies of the biodegradation of petroleum hydrocarbons – and in particular, naphthenic acids (NAs) – have focused on the role of bacteria, fungi, and emergent macrophytes. Algae have the possibility to become an important part of a biodegradation program. In addition to direct biodegradation, photosynthetic reactions intrinsically release oxygen into water, aerating it and promoting an environment for aerobic reactions. In order to utilize both algal biodegradation and community level effects, however, a greater understanding must be reached of the interaction of algal communities and oil-sands process-water. One of the algal species identified in oil-sands process-waters is *Chlamydomonas frigida*, which grows up to 10-20 ppm NA; while there is little information in the literature regarding this species, the genus is well-understood due to *C. reinhardtii*'s frequent use as a model organism. To begin understanding how green algae can tolerate NA contaminated water we performed a dose-response test with a wild type strain of *C. reinhardtii*. We found that it could grow in the presence of 100 ppm NA but observed that following 24 h of exposure to 100 ppm NA, the cells formed large clumps. The cells later grew as individual cells, albeit at a lower rate than untreated cells. We hypothesized that this clumping was due to interactions of NA with the glycoprotein cell wall of *C. reinhardtii*. To test our hypothesis we performed the same dose-response treatment with two cell-wall deficient, mutant strains of *C. reinhardtii*. While treatment with 100 ppm of NA slightly decreased the growth rates of both cell-wall deficient strains, we did not observe the same clumping phenomenon. To explore how NA interacts with the algal cell wall we examined NA treated cells of each strain using infrared spectromicroscopy and confocal laser scanning microscopy. We will present our data suggesting that NAs can suppress algal growth by directly interacting with the cell wall and causing the cells to sediment out of solution.

**WP036 Evaluating Sub-lethal Effects of Oil Sands Process-affected Waters on the White Sucker (*Catostomus commersonii*)** C.J. Arens, Univ of Prince Edward Island, Dept of Biomedical Sciences; N.S. Hogan, Canadian Rivers Institute, Univ of Prince Edward Island, Dept of Biology; G.J. Van Der Kraak, Univ of Guelph, Dept of Integrative Biology; M.R. van den Heuvel, Univ of Prince Edward Island, Dept of Biology. Conventional surface oil sands mining and extraction produces large volumes of tailings and oil sands process water (OSPW) that, under a zero-discharge policy, must be safely incorporated into a reclaimed landscape. One reclamation strategy under consideration is the development of end-pit lakes wherein microbiological processes detoxify tailings over time, improving water quality and allowing the base of a lake ecosystem to develop. However, there is evidence that fish exposed to OSPW exhibit disease pathologies and other signs of sublethal toxicity. The objective of our research was to determine the extent of potential impacts OSPW in end-pit lakes may have on the health of a native fish species, white sucker (*Catostomus commersonii*). In May 2010, two experimental ponds, Demonstration Pond and Bison Pond, containing mature fine tailings and lean oil sands material respectively, were each stocked with 200 white sucker from a local reservoir. In September 2010, 20 male and 20 female fish were sampled from each of these ponds, as well as the source population and two additional reference populations. Several endpoints were examined including disease occurrence, somatic indices, age

and growth relationships, immunological parameters, histopathology, as well as toxicant accumulation within tissues (e.g., naphthenic acid). Results indicated that fish introduced to Demonstration Pond exhibited a significant reduction in male gonad size, though this was not paralleled by decreases in sex steroid hormones. The Demonstration Pond population showed a reduction in growth relative to other populations sampled. Several other endpoints did not indicate detrimental impacts over a four month period. Bile fluorescence measurements showed a gradient of oil sands-associated exposure with the two experimental ponds being elevated over the source population of sucker, and over other local sucker populations. The results of this work allow us to develop an understanding of the potential impact oil sands tailings have on wild fish populations, as well as guide decisions regarding introduction and monitoring of fish in full scale end-pit lakes.

**WP037 Accumulation and Biliary Excretion of Naphthenic Acids in Yellow Perch Exposed to Oil Sands Influenced Waters** G.Z. MacDonald, Canadian Rivers Institute, Univ of Prince Edward Island; N.S. Hogan, Canadian Rivers Institute, Univ of Prince Edward Island, Dept of Biology; P. Fedorak, R. Young, Univ of Alberta; M.R. Vandenneuvel, Canadian Rivers Institute, Univ of Prince Edward Island. The major organic contaminant in waters influenced by oil sands mining are naphthenic acids. Naphthenic acids are a chemically complex group of compounds and the unavailability of analytical techniques confounded by their biotransformation in the environment has limited our understanding of the environmental fate of these compounds. Muscle tissue accumulation and biliary excretion of naphthenic acids was examined in yellow perch exposed to oil sands-influenced surface waters for 4 months. Pond stocking experiments with yellow perch were conducted in 2009 in two experimental ponds. One pond, the Demonstration Pond contained oil sands fine tailings capped with natural surface water and the other location, the South Bison Pond. Those ponds are currently known to contain 15 and 4 mg/L total naphthenic acids. Yellow perch were captured from a nearby reservoir, Mildred Lake, and placed in the two experimental systems. Two natural lakes, one at the edge of the Athabasca oil sands deposit, and one removed from the formation were also used for comparison purposes. Yellow perch dorsal muscle tissue was extracted and naphthenic acids were analyzed using GC-MS. A new method was adapted for the analysis of naphthenic acids in yellow perch bile involving the basic ethanolsis of conjugates, pH adjustment, solvent extraction, derivatization, and analysis by GC-MS. Duplicate bile extracts were analyzed by HPLC fluorescence at phenanthrene wavelengths. Perch tissue showed no accumulation of naphthenic acids in any of the five perch populations examined. In contrast, there was a clear gradient of naphthenic acids in the bile of perch, consistent with the water concentrations. Fluorescent metabolites were highly correlated with the bile naphthenic acid constituents, indicating that either the naphthenic acids have a fluorescent components, or that they are highly correlated with other aromatic constituents associated with oil sands. This study has demonstrated for the first time that weathered oil-sands derived naphthenic acids do not significantly accumulate in tissue, and are rapidly excreted in bile, making the analysis of naphthenic acids in bile a useful indicator of exposure to oil sands-impacted waters. The nature of the aromatic component within the mixture will be the subject of further studies.

**WP038 Weight of Evidence Approach to Assess Metal Cycling in Oil Sands Tailings and Natural Environments – 1. Metals in Water** P. Huntsman-Mapila, Natural Resources Canada; N. Puttaswamy, R. Cameron, M. Pawlak, Y. Thibault, C. Rickwood, Natural Resources Canada. A project to examine the fate and effect of thirteen priority metals in oil sands tailings and the Athabasca River was launched by Natural Resources Canada in 2010 with the first round of samples collected in Spring 2011. The overall investigation into fate measures metal concentrations and assesses the abiotic and biotic factors influencing their fate in various environmental compartments (water, sediment and suspended solids). This presentation outlines the results of the first round of snow and river samples collected from both the Athabasca and Muskeg River area in Spring 2011. To link with the effects component of the project, metal bioavailability was also examined through laboratory speciation analysis of various metals and the deployment of diffusive gradient thin film (DGT) devices in the river for a period of 10 days.

**WP039 Weight of Evidence Approach to Assess Metal Cycling in Oil Sands Tailings and Natural Environments – 2. Metals in Sediment** P. Huntsman-Mapila, Natural Resources Canada; S. Langley, N. Puttaswamy,

Y. Thibault, C. Rickwood, Natural Resources Canada. A project to examine the fate and effect of thirteen priority metals in oil sands tailings and the Athabasca River was launched by Natural Resources Canada in 2010 with the first round of samples collected in Spring 2011. The overall investigation into fate measures metal concentrations and the abiotic and biotic factors influencing their fate in various environmental compartments (water, sediment and suspended solids). This presentation outlines the results of the first round of sediment samples collected from both the Athabasca and Muskeg River. Sediment cores collected at 6 sites on the river were sectioned and examined for sediment and pore water metal concentrations. Microcosm studies were performed to elucidate potential links between microbial respiratory processes and metal release from sediments. Sequential extractions of the metals in the sediments and sediment mineralogical characterization were also undertaken to identify the phases in which the metals of interest reside. Results of the sediment analyses are linked to the sediment toxicology component of the project.

**WP040 Weight of Evidence Approach to Assess Metal Cycling in Oil Sands, Tailings, and Natural Environments – 1. Exposure to Water** C. Rickwood, Natural Resources Canada, CANMET MMSL, Univ of Saskatchewan, Dept of Toxicology; M. Desforges, M. King, Natural Resources Canada, CANMET MMSL; N. Puttaswamy, Natural Resources Canada; P. Huntsman-Mapila, Natural Resources Canada, CANMET-MMSL. An investigation was conducted to assess the distribution and fate of metals in oil sands tailings streams and adjacent natural rivers. Based on recently published work regarding metals in the local environment, this project aimed to assess both the fate and effect of thirteen priority metals. The investigation into fate measured the type of metals and the abiotic and biotic factors influencing their fate in various environmental compartments (water, sediment and suspended solids). The investigation into effect used various toxicity testing methods to assess the impacts of both natural rivers and tailings ponds water, fluid fines/suspended sediments and sediments. This presentation outlines the results of the impact assessment of the water exposure component conducted during the first round of testing with samples collected in spring 2011 from both the Athabasca and Muskeg River. Tests were conducted with algae (*Pseudokirchneriella subcapitata*), invertebrates (*Ceriodaphnia dubia*) and fish species (*Pimephales promelas*). Metal bioavailability was assessed in conjunction to the metal data collected during the fate portion of the project (presented elsewhere). Using a weight of evidence approach it is possible to investigate the fate of metals and their potential effect on aquatic organisms from water samples in the Athabasca river region.

**WP041 Weight of Evidence Approach to Assess Metal Cycling in Oil Sands Tailings and Natural Environments – 2. Exposure to Sediments** C. Rickwood, Natural Resources Canada, CANMET MMSL, Univ of Saskatchewan, Dept of Toxicology; N. Puttaswamy, M. Desforges, O. Samotus, P. Huntsman-Mapila, Natural Resources Canada. An investigation was conducted to assess the distribution and fate of metals in oil sands tailings streams and adjacent natural rivers. Based on recently published work regarding metals in the local environment, this project aimed to assess both the fate and effect of thirteen priority metals. The investigation into fate measured the type of metals and the abiotic and biotic factors influencing their fate in various environmental compartments (water, sediment and suspended solids). The investigation into effect used various toxicity testing methods to assess the impacts of both natural rivers and tailings ponds water, fluid fines/suspended sediments and sediments. This presentation outlines the results of the impact assessment of the sediment exposure component conducted during the first round of testing with samples collected from both the Athabasca and Muskeg River in Spring 2011. Tests were conducted using benthic invertebrate (*Chironomus dilutus*) and fish species (*Pimephales promelas*). Metal bioavailability was assessed using peepers and DGTs in sediments and compared to the metal data collected during the fate portion of the project (presented elsewhere). Using a weight of evidence approach it is possible to investigate the fate of metals and their potential effect on aquatic organisms from Athabasca river region sediment samples.

**WP042 Assessment of the Aquatic Toxicity of Waters from Oil Sands Tailings Rapidly Dewatered Using Atmospheric Fines Drying Process** S. Armstrong, Shell Health – Americas; J. Reid, Shell Canada Ltd. The following research investigated the aquatic toxicity of release waters from Shell's pilot atmospheric fines drying (AFD) process from its oil sands operation and compared it to the reclaim waters of untreated mature fine tailings

(MFT). The AFD tailings treatment process is essential technology developed to rapidly release water from oil sands tailings that would otherwise be trapped in traditional tailings deposits so that these waters may be recycled back into the extraction process. The objective of this research is to verify that the pilot AFD release waters have not increased toxicity or environmental risk compared to the current MFT and associated waters. AFD and MFT waters were tested for acute toxicity using 96 h rainbow trout (*Oncorhynchus mykiss*) bioassay and the Microtox® (*Vibrio fischeri*) bacterial luminescence acute toxicity bioassays. Chronic toxicity was investigated using Fathead minnow (*Pimephales promelas*) 7-day chronic bioassay and the freshwater invertebrate (*Ceriodaphnia dubia*) 7-day chronic reproduction bioassay. There was no acute toxicity differences between MFT reclaim water and AFD release waters in fish bioassays for lethality and growth. Toxicological effects due to oil sands tailings waters were only observed in the reproduction of the freshwater invertebrate *Ceriodaphnia dubia* after chronic exposure. There was no increased toxicity observed in the AFD compared to that of the MFT reclaim waters. The inhibition to reproduction in *Ceriodaphnia* was moderate and comparable for AFD and MFT treatments. Based on the results collected to date, the AFD release water has no increased inherent hazard for acute toxicity and chronic toxicity over current MFT reclaim water. The long term stability of the AFD tailings after drying and rewetting and overwintering is still unknown and is the subject of ongoing research. These current results indicate that AFD tailings treatment is a suitable tailings treatment strategy for MFT and will assist oil sands operators in meeting the Alberta Environment's Directive 74 to produce solid trafficable tailings.

**WP043 Application of Ozone/Hydrogen Peroxide Advanced Oxidation Process for Treatment of Naphthenic Acids in Oil Sand Process Water** A. Afzal, P. Drzewicz, Univ of Alberta, Civil and Environmental Engineering;

J.W. Martin, Univ of Alberta, Dept of Laboratory Medicine and Pathology; M. Gamal El-Din, Univ of Alberta, Civil and Environmental Engineering. The oil sands deposits of Northern Alberta, Canada contain approximately 1.7 trillion barrels of oil in form of bitumen, from which crude oil is extracted using surface mining method. For every 1 barrel of oil produced, between 2 and 5 barrels of freshwater are required. This high amount of oil sand process water (OSPW) is acutely toxic and contained in large tailing ponds due to zero discharge policy. Naphthenic acids (NAs) are believed to be the most significant toxic components in oil sand deposits and tailing ponds. Biodegradation which occurs naturally in tailing ponds is too slow to be an effective remediation technology for NA removal from OSPW. Advanced oxidation processes are among techniques that can be used for degradation of refractory NAs. In this study combination of ozone ( $O_3$ ) and hydrogen peroxide ( $H_2O_2$ ) was used for decomposition of NAs in OSPW. In our research experiments, in a semi-batch reactor with 4 L capacity,  $O_3$  was bubbled to the OSPW and the NA concentration was measured using Fourier Transform Infrared Spectroscopy (FT-IR) before and after treatment.  $H_2O_2$  with different molar ratios to  $O_3$  was added to the OSPW right before the ozonation. Results of a control experiment showed that after 4 min ozonation without  $H_2O_2$  addition, 29% of NAs was removed. The  $O_3$  concentration was 30 mg/L and the NA concentration was 70 and 50 mg/L before and after treatment, respectively. However with adding the  $H_2O_2$  at molar ratios of 1:1, 1:2, and 2:1 to  $O_3$ , the degradation was increased by 39, 39, and 37 %, respectively. The reason is that  $O_3$  in combination with  $H_2O_2$  generates an OH radical which is a strong oxidizing agent that can oxidize the NAs to more biodegradable compounds such as hydroxyl-NAs. It is expected that the optimized method with the higher ozone and  $H_2O_2$  concentrations is promising for NAs removal in OSPW.

**WP044 Toxicity Reduction of Oil Sands Process Affected Water by Native Phytoplankton Species** A.P. Woodworth, Univ of Waterloo, PhD

Candidate; A. Bauer, Univ of Waterloo, Dept of Biology; R.A. Frank, Environment Canada, AEPRD, Univ of Guelph, Dept of Env. Biology & Toxicology; K.M. Muller, B. McConkey, Univ of Waterloo, Dept of Biology. Naphthenic acids (NAs), a class of compounds resulting from bitumen extraction processes, have been identified as one of the principal toxic components of oil sands process-affected water (OSPW). NAs are known to be acutely toxic to many organisms including fish and aquatic invertebrates, but are considerably less toxic to many algal species. Due to the toxic nature of NAs, they have recently been the focus of several studies investigating potential remediation strategies. One such investigation, involving the accumulation or adsorption of NAs by phytoplankton strains isolated from the Athabasca oil sands region, has recently identified three phytoplankton

strains capable of reducing total naphthenic acid concentrations in an oil sands naphthenic acid mixture. This study examines the reduction of NA mixture toxicity to the aquatic invertebrate *Daphnia magna* by the three phytoplankton strains. The effects of phytoplankton culture density and nitrogen limitation on the capacity of algae to reduce NA mixture toxicity were also examined. Axenic phytoplankton cultures were exposed to a 100mg L<sup>-1</sup> mixture of NAs for a total of 14 days. The resulting algal biomass was then removed using centrifugation followed by vacuum filtration and the filtrate retained for toxicity testing. Forty- eight hour acute lethality tests were then performed on *D. magna* neonates, and the relative toxicity of the NA mixtures before and after algal treatment were compared. Preliminary results indicate algae species were effective at reducing NA toxicity, and the toxicity reduction is correlated to phytoplankton culture density.

**WP045 Understanding Interactive Effects of Climate Change and Toxicity: Cadmium Reverses Beneficial Effects of Temperature in a Freshwater Snail** D.A. Kimberly, Texas Tech Univ/TIEHH, Environmental

Toxicology, Texas Tech Univ, Environmental Toxicology; C.J. Salice, Texas Tech Univ/TIEHH, Environmental Toxicology, Assistant Professor, Texas Tech Univ. The goal of ecological risk is to protect ecosystems against the adverse effects of toxicants. In practice, ecological risk assessments are frequently based on data in which study organisms are exposed to a single stressor only. However, natural populations of organisms invariably experience multiple, simultaneous stressors. As an example, many aquatic systems experience a wide range of temperatures which may expand given projected changes in climate. Hence, ecological risk assessments of chemicals should consider the impacts of higher and more variable temperatures on aquatic species. The objective of this study was to characterize the interactive effects of cadmium and temperature stress on development and hatching of eggs from the freshwater snail, *Physa pomilia*. Ninety six egg masses were collected from 20 breeding pairs and evenly divided between two temperatures of 25C and 35C and among four cadmium treatments of 0, 5µg/L, 15µg/L, and 25µg/L for a total of eight treatments. The temperatures represent a normally occurring (25C) and a higher temperature that may be expected under climate warming scenarios (35C). Egg masses were observed twice daily for days-to-hatching and hatching success. Overall, egg masses reared at 35C and exposed to Cd had significantly lower hatching success and greater time-to-hatching, compared to the control. In fact, control egg masses at 35C had a slightly higher hatching success in the absence of cadmium compared to controls at 25C. Additionally, time to hatch was lower at 35C compared to 25C in the absence of cadmium. It was only when egg masses were exposed to temperature and cadmium in combination that adverse effects of higher temperature were observed. Climate change is a factor that may be highly variable in the field and is of major importance to the physiological state of organisms. Our data suggest that, in some cases, adverse effects of climate change may only exist in the presence of certain other stressors. Specifically in our study, an increase in cadmium exposure concomitant with high temperatures lead to adverse effects in freshwater snails. Thus, ecological risks associated with climate change must be assessed in the context of other stressors such as pollutants as the combined exposure may dwarf or intensify overall adverse effects. Future efforts should also focus on how biological stressors such as competition and predation may interact with chemical stressors in a changing climate.

**WP046 Preliminary Model Prediction of Climate Change Impact on the Multimedia Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in South Korea** J. Song, D. Lee, S. Kim, Y. Lee, Seoul National

Univ, Dept of environmental planning, Graduate school of environmental studies. A dynamic multimedia model was developed and evaluated to predict the change in multimedia distribution of semi-volatile organic compounds by taking into account the effects of climate change (CC) including those of temperature and other meteorological and environmental conditions such as wind speed and direction, precipitation rate, radiation intensity, hydroxyl radical concentration, particulate matters and suspended solids levels, vegetation, and land cover. The model is capable of predicting the time change of the concentrations in air, water, soil, and vegetation in the entire territory of South Korea (34N-39N, 125E-130E). A total of 176 air grids and 98941 watersheds were within the model domain. Four vertical layers were used to describe the vertical concentration variation in air. The model was evaluated by comparing the predicted relative concentrations with the monitoring data (4) from 1999 to 2003 for 15 PAHs. From the simulations extended for a 30 year period from 2000 to 2030, the relative



concentrations of water/air, soil/air, vegetation/air, water/soil, and sediment/water were compared between BAU and A1B scenarios. Five different cities chosen for the comparisons were Seoul, Kwangwon, Chungbuk, Kwangju, and Pusan. Emission rates were set identical in the two scenarios to explore the impacts of CC alone. The impacts of CC on the relative concentrations varied with the physicochemical properties of the PAHs. The direction of the impacts also changed with the locations. For example, under the A1B scenario, the relative benzo[a]pyrene concentration of water/air and water/soil increased in all locations while soil/air, vegetation/soil, and sediment/water decreased in some of the locations. It was also noted that the fluctuation of the concentrations or relative concentrations substantially increased under the A1B scenario due to more frequent occurrence of extreme weather conditions. More details of the simulation results and the contributing factors to the CC impacts will be presented. The model prediction strongly suggests that CC will change the distribution of PAHs among the environmental media, which may lead to increased risk to human or ecosystem even when the emission rate is kept constant or reduced. Further studies are warranted to predict the direction and the magnitude of such changes and to reduce the uncertainties associated with the predictions.

**WP047 Maternal Transfer of MeHgCl in Northern Water Snakes, *Nerodia sipedon* and its Effects on Neonate Performance** R.C. Wright, J.W. Cusack, C. Henry, F.C. Bailey, Middle Tennessee State Univ, Biology. In ecotoxicology, reptiles have historically been underrepresented, with most studies focusing on the concentration of a contaminant found in an organism at a site. Fewer studies have been conducted to examine the fate or physiological/biochemical effects of contaminants on these animals. This study was designed to look at the effects of maternally-transferred MeHgCl on locomotor performance in Northern Water Snake (*Nerodia sipedon*) neonates. In the study, adult female *N. sipedon* were randomly assigned to one of three treatments: 0, 10, or 10,000 ug/Kg. Fourteen females gave birth and after a two week acclimation period the neonates were tested for average and maximum terrestrial locomotor speed in a laboratory race track. Mercury analyses of neonate livers indicated that neonates from the high dose treatment had the highest concentration of Hg. No statistical difference was found in speed of locomotion between treatments (ANOVA,  $F_{2,11} = 0.021$ ,  $p = 0.980$ ).

**WP048 The Relative Importance of Diet and Dermal Contaminant Exposure in Reptiles: Results from Empirical Studies on the Western Fence Lizard** S. Weir, Texas Tech Univ, The institute of environmental and human health; L. Talent, Oklahoma State Univ; C. Salice, Texas Tech Univ, The institute of environmental and human health. Reptile ecological risk assessment is currently limited by a lack of acute and chronic toxicity data as well as a dearth of knowledge regarding contaminant exposure. Recent advances in the use of the western fence lizard (*Sceloporus occidentalis*) as a model organism creates opportunities for filling in important data gaps that may improve reptile risk assessment. Of particular interest is in understanding which exposure routes contribute most to total exposure. Because many reptiles spend a large proportion of time in contact with substrate, there is potential for dermal exposure to be important for reptiles; dermal exposure is known to be important for birds. We conducted a study to provide insight into the relative importance of diet and dermal contaminant exposure in reptiles. Fence lizards were exposed to phthalate esters by either oral gavage or dermal application and we analyzed liver and body fat for phthalates at 3 time points following exposure. Phthalates were chosen as model chemicals because lipophilicity changes with the length of the side chain allowing relationships between dermal exposure and lipophilicity to be estimated. We used di-methyl phthalate (DMP, logKow = 1.6), di-butyl phthalate (DBP, logKow = 4.5), and di-n-octyl phthalate (DNOP, logKow = 8.0). Our results suggest that dermal exposure increases with increasing lipophilicity and is generally sequestered into fat tissue rather than liver. Of the phthalates tested, DMP did not show significant tissue concentrations via the dermal route, and also relatively low concentrations via oral exposure as well. DBP appeared to be significantly absorbed by both dermal and oral exposure, with oral exposure generally resulting in greater tissue concentration than dermal exposure. Finally, DNOP was very highly absorbed via both oral and dermal exposure routes, with little difference in tissue residues between the two. Our results highlight the potential importance of dermal exposure as a significant exposure route, especially for reptiles. Future research will further quantify the relative importance of oral and dermal exposure routes under more realistic scenarios including contaminated soil and food items. We

suggest that ecological risk assessments involving reptiles should consider the dermal exposure route, especially because dietary exposures may be relatively low due to low metabolic demands.

**WP049 Organotypic Cultures in Protected Wildlife: Applications in the Toxicology of Chemical and Natural Stressors in Sea Turtles** J. Cole, S. Wiggins, G.V. Zychowski, The Institute of Environmental and Human Health, Texas Tech Univ, Dept of Environmental Toxicology; B. Higgins, NOAA/NMFS; J. Strahlendorf, Texas Tech Univ Health Sciences Center Dept of Cell Physiology and Biophysics; J. Olson, Univ at Buffalo SUNY; C. Godard-Coddington, The Institute of Environmental and Human Health/Texas Tech Univ, Assistant Professor, Texas Tech Univ/The Institute of Environmental and Human Health, Assistant Professor. Sea turtle health assessment is crucial to conserving declining sea turtle species and evaluating contaminant impacts on ocean life. All seven species of sea turtles are protected under the Endangered Species Act. Because they are protected, researchers are challenged to develop minimally-invasive methods to detect and evaluate contaminant impacts. To address this issue, we developed a methodology pairing organotypic cultures of precision-cut slices prepared from sea turtle skin biopsies with gene, protein and enzymatic analyses. Here, we exposed organotypic cultures of loggerhead sea turtle (*Caretta caretta*) skin tissue obtained from biopsies to a dose range (0, 0.01, 0.1, 1 and 10 uM) of benzo[a]pyrene (BAP), a prototypical polycyclic aromatic hydrocarbon and known marine contaminant. We evaluated the viability of the organotypic culture slices after 24 and 72 h by measuring intracellular potassium concentrations of control slices using a Flame Atomic Absorption Spectrometer. As expected, viability was observed in control cultures at all time points. Protein expression analyses were optimized on membrane bound protein lysates after tissue slices were homogenized using a mortar and pestle, liquid nitrogen and ultrasonication. Western blot analysis showed CYP1A-like activity measured in BAP-induced organotypic cultures using a polyclonal goat IgG anti-rabbit P4501A1/2 antibody. Gene expression and enzymatic studies using qRT-PCR and a kinetic catalytic assay, respectively, are underway. Our preliminary results and ongoing analyses indicate that precision-cut organotypic cultures studies are promising tools for 1) studying the impacts of contaminants on marine reptiles at the molecular level 2) investigating impacts of toxic chemical exposure and natural stressors on marine reptiles and 3) identifying and validating molecular changes as biomarkers of pollution impacts on marine reptiles and other wildlife.

**WP050 Research on the Migration of Young Green Turtles (*Chelonia mydas*) in the ArcelorMittal Tubarão Industrial Effluent** J.O. Santos, ARCELORMITTAL TUBARÃO, Environment; F. Passamani, ARCELORMITTAL TUBARÃO, Environment, ArcelorMittal Tubarão, Environment; C. BAPTISTOTTE, E. TOREZANI, TAMAR-ICMBIO. The green turtle, *Chelonia mydas*, is globally distributed in tropical and subtropical seas. In Brazil, their reproduction areas are located in the oceanic islands of Fernando de Noronha, Atol das Rocas and Trindade Island. Young and adult specimens are found in feeding areas along almost the entire coast of Brazil. This species, as well as the other four species existing in the Brazilian coast, is part of the list of endangered fauna species in Brazil, also considered endangered by the World Conservation Union (Hilton-Taylor 2000). The research institution – Projeto TAMAR-IBAMA – has acted especially on the main reproduction areas in Brazil since 1980, and in 1990 it started working in the feeding areas. Biometric and marking works are carried out in the reproduction and the feeding areas by the institution, aiming to better understand the biological and migration standards of this species, which serve as basis for preservation activities (Marcovaldi et al. 1998). In 2000 a technical cooperation agreement was signed between the research institution and ArcelorMittal in order to carry out studies on the biology of the species in the area of influence of the company. Field works have been performed since August 11th, 2000 with the main objective of carrying out biometric studies by capturing and marking them to record growth, migration patterns, haematological profile and health conditions of the green turtles that use the marine water of ArcelorMittal Tubarão effluent area. ArcelorMittal Tubarão is located 14km north of Vitória (State of Espírito Santo, Brazil). Treated domestic and industrial effluents from the company are thrown in one single marine area (final effluent). Water and sediments receive physical-chemical and toxicological monitoring every six months and a biological monitoring is also carried out examining the bioaccumulation of heavy metals in bivalve molluscs. The area studied is located at the end of the effluent channel and has an average temperature 6° above sea water temperature, which, together

with the availability of organic matter, favours an environment rich in algae. This study presents the monitoring results from 2009-2010 including field methods and data collection results from the effluent area.

**WP051 Influence of Atrazine Utilization in Paddy Field on Morphology of the Populated Rice Frog *Fejervarya limnocharis* in Thailand** P. Tham-machori, W. Khonsue, Chulalongkorn Univ, Dept of Biology; J. Kitana, Chulalongkorn Univ, Dept of Biology, Faculty of Science; P. Varanusupakul, Chulalongkorn Univ, Dept Chemistry; N. Kitana, Chulalongkorn Univ, Dept of Biology. Intensive herbicide utilization in agricultural area can lead to an environmental contamination and adverse effects on non-target organisms living in the area including amphibians. In northern part of Thailand, there are many agricultural areas where herbicide, especially atrazine, has been used for a long time. To test for atrazine contamination, environmental samples (soil and water) were collected from a potentially contaminated agricultural area with intensive atrazine usage and a reference site with no history of atrazine usage in Nan province, northern part of Thailand during 2010-2011. The results of gas chromatography-mass spectrometry analysis showed that atrazine can be found in low level (0.15 mg/L) in water of the potentially contaminated paddy field, but not in the reference paddy field. To test for influence of atrazine contamination on non-target organisms, the rice frog *Fejervarya limnocharis* was used as a sentinel species for environmental health hazards. Frogs were field-collected during July 2010 to June 2011 from the contaminated site and the reference site. Frogs were subjected to contaminant analysis for atrazine by high performance liquid chromatography, and morphometry and gravimetry of liver, kidney, gonad and body. Site-related difference on tissue residue of atrazine and parameters of morphometric and gravimetric analyses will be presented and discussed. Since atrazine is known as endocrine disrupting chemicals, potential impacts of its contamination on reproductive and fecundity fitness of this populated frog species will be evaluated. The results of this study could be used as an early warning of environmental health hazards of atrazine utilization in other vertebrates living near the agricultural areas including human.

**WP052 Effects of Pond Sediment Exposure on Steroidogenesis in Juvenile Cane Toads (*Bufo marinus*)** D. Fort, Fort Environmental Laboratories, Fort Environmental Laboratories, Inc.; C. Fort, B. Todhunter, Fort Environmental Laboratories, Inc.; J. Bacon, Bermuda Zoological Society. The impacts of contaminated sediment from two ponds in Bermuda on early sexual differentiation and gonadal steroidogenesis in juvenile cane toads were examined. Pond sediment from Bermuda was previously found to cause reduced reproductive fecundity and alter steroidogenesis in fathead minnow (*Pimephales promelas*) and killifish (*Fundulus heteroclitus*). A partial lifecycle study exposing Gosner stage 20 cane toad tadpoles to pond sediment and laboratory culture water through metamorphosis and into a juvenile state was performed. An increase in phenotypic males was noted in the pond sediment treatments; however, no effects on survival, metamorphic completion, body weight, snout-vent length, or GSI were noted. To further examine the effect of pond sediment exposure on gonadal differentiation aromatase and CYP 17 lyase (CYP 17) were measured; in addition to plasma estradiol (E2), testosterone (T), dihydrotestosterone (DHT), and vitellogenin (VTG) levels in post-metamorphic toads. Gonad and plasma samples were collected from juvenile control specimens and organisms exposed to pond sediment from early embryo through metamorphosis. Increased mean plasma DHT and T, and decreased mean E2 and VTG levels were found in juvenile toads from the pond sediment treatment compared to controls. Toads exposed to the pond sediment exhibited a decrease in mean gonadal aromatase activity, but CYP 17 lyase activity was unaffected relative to control toads. These results are consistent with similar studies conducted in fish and suggest that the decreased aromatase activity may have resulted in the increase in plasma T and DHT levels found in pond sediment-treated toads. Consistent CYP 17 activity suggested that upstream androgen biosynthesis was not affected by pond sediment exposure. Further, the increase in the proportion of phenotypic male frogs may be the result of increased gonadal androgen levels induced by exposure to pond sediment contaminated with petroleum hydrocarbons, polycyclic aromatic hydrocarbons, and metals.

**WP053 Influence of Roadway Proximity on Mercury Accumulation and Population Dynamics in a Wetland-dwelling Amphibian** J. Bushey, Univ of Connecticut, Asst Professor; S. Brady, Yale Univ, Ecology & Evolutionary Biology; A. Aragon-Jose, Univ of Connecticut; D. Skelly, Yale Univ. Roads are distributed pervasively throughout much of North America. Runoff

from impervious surfaces contributes trace metal contaminants to roadside wetlands. However, the impact of salinization of waterways on biogeochemical cycles, particularly organic carbon cycling, and Hg speciation is lacking. DOC and Hg-Cl complexes impact potential bioavailability, particularly at sub-lethal levels. Many investigations focus on lethal doses of single chemical exposure. However, our burgeoning understanding of the influence of rapid evolution on ecological patterns suggests that sub-lethal geochemical processes and demographic processes may interact, resulting in outcomes that vary among local populations. We examined the potential influence of road proximity on the chemical characteristics of five forest and five roadside temporary wetlands located in northeastern Connecticut. In roadside wetlands, specific conductance—a close proxy for road salt—was approximately 25 times that of forest pools, demonstrating the influence of impervious road surfaces. Hg species along with base cation, anion and DOC content were analyzed from water samples collected in April, May and early July 2009. To investigate biological outcomes, we used a reciprocal transplant experimental design in which spotted salamanders embryos were grown out in field enclosures in each of the ten wetlands to parse the influence of the environment and the population on bioaccumulation. Total Hg values in late-stage larvae in roadside ponds increased 25% relative to forested ponds. Average larval content was 0.10 ppm with surprisingly lower levels for roadside ponds relative to forested ponds, possibly reflecting the decrease in dissolved organic carbon. Additionally, we examined the potential impact of maternal transfer on the reproductive success of amphibians. We collected adult woodfrogs in Spring 2010 returning to select roadside and forested vernal pools in the Yale Experimental Forest, CT. Pairs of amphibians were bred in captivity with mating adults and a subsection of the fertilized eggs analyzed for Hg content. Reproductive success was documented by hatchling success. Adult females contained higher Hg concentrations (176 ng/g) relative to males (130 ng/g), with slightly higher content in forest specimens. While egg content increased with female parent Hg content for both roadway and forest specimens, maternal transfer of Hg was only significantly related ( $p=0.05$ ) for the forested samples.

**WP054 Occurrence of Current Use Pesticides in Amphibian Habitats in Northern California** K.L. Smalling, US Geological Survey; G. Fellers, P. Kleeman, US Geological Survey, Western Region Ecological Center; M. McWayne, US Geological Survey; W. Battaglin, US Geological Survey, Denver Federal Center; K. Kuivila, US Geological Survey. Amphibian populations worldwide have been in decline for at least the past two decades. Habitat loss, predation, disease—particularly the proliferation of the chytridiomycosis causing fungus, *Batrachochytrium dendrobatidis* (Bd)—and pollution, including pesticides, are factors that have been implicated. Surveys of natural populations have shown correlations between population declines and proximity to agricultural lands. Pesticides are receiving increasing attention as a potential cause of amphibian declines, acting singly or in combination with other stressors. This study focuses on the potential exposure and accumulation of pesticides in pond-breeding frogs collected from 11 sites in Northern California. Water, sediment and frog tissue were analyzed for over 90 current-use pesticides and pesticide degradates including 34 fungicides by using gas chromatography mass spectrometry. Eighteen pesticides were detected in water and sediment samples. The herbicide, glyphosate, and its primary degradate, AMPA, were detected most frequently (11 and 15 %, respectively) and at the highest concentrations in water while fungicides, such as tebuconazole and pyraclostrobin, were most frequently found in sediment (40 % detection frequency). Preliminary results suggest that amphibians are exposed to a variety of pesticides in both water and sediment and fungicides represent a substantial portion of the potential pesticide exposure. Tissue analysis is ongoing; however, previous studies report detections of organophosphate insecticides including chlorpyrifos in amphibians collected from similar sites in Northern California. Limited information is available on the accumulation of pesticides and pesticide degradates, particularly fungicides, in amphibians as well as the degree to which these agents may be playing a role in the loss of amphibians worldwide.

**WP055 Assessment of the Endocrine Toxicity of the Fungicide Prochloraz Using the Larval Amphibian Growth and Development Assay** A. Olmstead, US Environmental Protection Agency, Mid-Continent Ecology Division; L. Blake, P. Kosian, J. Haselman, J. Korte, R. Johnson, C. Blanksma, K. Lillegard, S. Zavodnik, B. Heggestad, S. Degitz, US Environmental Protection Agency. Prochloraz is a broad spectrum fungicide that acts by inhibiting ergosterol biosynthesis in target species. Toxicity results

in non-target vertebrate species suggest this toxicant acts as an endocrine disruptor that inhibits aromatase, the enzyme responsible for the conversion of androgens into estrogens. We used the Larval Amphibian Growth and Development Assay, a Tier II test in the USEPA's Endocrine Disruptor Screening Program to examine the effects of prochloraz on the model amphibian species, *Xenopus laevis*. Larval *X. laevis* were exposed from just after fertilization until nine weeks after completion of metamorphosis in a flow-through diluter system. Exposed animals were assessed at completion of metamorphosis and at the end of the exposure for endocrine-related endpoints as well as general measures of toxicity. No effects on mortality, growth, or metamorphosis times were observed. Using genetic sexing, no sex reversals among exposed individuals were observed. Both females and males had reduced liver somatic indices at exposure concentrations of 60 and 180 µg/L respectively. Exposure to prochloraz concentrations of 60 µg/L and above resulted in very low, but significant induction of circulating vitellogenin. Observed effects indicate that prochloraz has some endocrine activity, however, these effects are different from those observed with the pharmaceutical aromatase inhibitor, fadrozole, suggesting that prochloraz is eliciting these effects with a different mode of action. In addition, these results illustrate the utility of the Larval Growth and Development Assay to evaluate endocrine toxicity of toxicants.

**WP057 Preliminary Histological Examination as a Means to Evaluate the Effects of Aqueous Pine Extract of Virginia Pine on African Clawed Frogs** L. Minton, J. Rayburn, M. Hamissou, G. Cline, Jacksonville State Univ, Biology Dept. Estrogenic compounds are introduced into the habitats of amphibians through a multitude of mediums, the majority of which are influenced or created due to human actions. Couple the reliability of amphibians as indicators with the ease to which they are exposed and the model genus of *Xenopus* becomes important test organisms for a wide array of environmental problems. Some naturally occurring estrogens found in the environment as compounds in some pine trees have been determined to cause estrogenic changes in fish and other organisms. For our test, we exposed groups of *X. laevis* in tanks to concentrations of aqueous pine tree extract (from Virginia Pine, *Pinus virginiana*). The long-term exposure experiment lasted 3.5 months, with individuals being exposed from 96hrs after fertilization until they were at approximately NF stage 64-66 (at metamorphosis), including the stage of gonadal differentiation. Once they reached this stage of development they were removed from the tanks, anaesthetized and fixed in formalin to await histological procedures. Dosages of the pine tree extract were administered on a biweekly basis using test concentrations of differing percentages (1% – high, 0.5% – low), and throughout the experiment, the frogs were kept in covered 20L tanks with a controlled temperature range of 25-29° Celsius. Effects from this pine tree extract were examined using time to metamorphosis, malformations, mortality, and histological observations. Treated individuals were compared to subjects in the positive control groups (exposed to estrogen) and negative control (no treatment). The study is focused on determining potential gonadal abnormalities caused by pine tree leachate exposure. These evaluations will focus on the testes and the ovaries, and will be examined using histological slides, staining, and light microscopy.

**WP058 Battle in the Bucket: Competitive Interactions Among Invasive Mosquitoes and Implications for the Potential Spread of Infectious Disease** M.K. Trawick, C.J. Salice, Texas Tech Univ, Dept of Environmental Toxicology. Invasive species cause significant ecological and economic damage and, in some cases, can represent a more direct risk to humans as vectors of infectious disease. Probabilistic risk assessments represent a cost effective and useful tool for maximizing resource allocation by policy makers and public health officials to minimize damage caused by invasive species. Mathematical models of ecological processes can help refine empirical studies by elucidating critical field parameters and can point to population level consequences of stage-specific management action. Additionally, well constructed models can assist in predicting how ecological interactions such as competition might change in novel locations or conditions, such as those predicted by climate change forecasts. Mosquito-borne zoonoses contribute to significant morbidity and mortality in developing nations, and have been predicted to increase their prevalence within the United States in the near future. The invasive mosquito *Aedes albopictus* competes with the major Dengue fever vector *Ae. aegypti* in the larval stage of development. Hence, ecological interactions among these species have important consequences for public health. To gain further insight into mosquito population dynamics

and competitive interaction, we designed a stage-specific discrete time mathematical simulation model that takes into account larval competition between these two species. Based on the projection matrix framework with five distinct live life stages and a two-day timestep, density dependence functions operate in two larval stages. Our modeling results support those of others in which mosquito population dynamics are driven strongly by the larval period. Additionally, our results indicate that species persistence is sensitive to the average larval survivorship and the magnitude to which species impact each other. Some research suggests that differential egg mortality under changing climatic conditions could explain the persistence of the inferior competitor *Ae. aegypti*, and we are currently incorporating this into the model. Furthermore, pesticide exposure has been shown to impact competitive interactions between invasive mosquito populations. To that end, we will supplement our model with empirical studies of the effects of target and non-target pesticides. The model presented in this abstract will ultimately be combined with forecasted climate change models to identify areas of high concern of disease transmission.

**WP059 Understanding the Spread of Invasives: Using Functional Traits to Guide Assessment and Management Efforts of Nonindigenous Species** C.J. Salice, Texas Tech Univ/TIEHH, Environmental Toxicology, Assistant Professor, Texas Tech Univ; S. Weir, Texas Tech Univ, The institute of environmental and human health; L. Beaty, Texas Tech Univ, Dept of Biology. Invasive species are a significant threat to ecological systems and the economy worldwide. Hence, there is a need to develop methods to identify, evaluate and reduce risks of invasion. Although the ecological risk assessment process has traditionally focused on chemical pollutants, there are elements that can be adapted to invasive species risk assessment. In particular, trait-based assessments may be well suited to evaluating risks associated with the spread of nonindigenous species. In effect, species' traits determine their invasion potential and success. Broadly, invasive species possess traits that (1) allow for enhanced growth and exploitation of resources and/or (2) allow for persistence under stressful conditions. By understanding which functional traits are characteristic of particular nonindigenous species, we may be better able to control invasions. Here, we present two case studies in which we evaluated the traits of different invasive species as part of ongoing risk assessments for both. The invasive red-rimmed melania, *Melanoides tuberculatus* is a slow-growing freshwater gastropod that is among the most invasive mollusks. We showed that this species is significantly more tolerant than native gastropods to a wide range of chemical and physical stressors imparting an advantage, especially in anthropogenically impacted sites. These data suggest that defensive traits may favor the invasion potential of *M. tuberculatus* but also provide insight into potential areas to which this species could spread. Alternatively, high reproductive and growth rates in cane toads, *Rhinella marina*, are clear contributors to the spread of this species in Australia. We used a series of stage-based and individual-based population models to explore the life history mechanisms by which this species obtains high population size and further used the models to help identify optimal control strategies. Control techniques that reduced survival of young toads had favorable management outcomes. In this case, identifying control strategies that target key traits (juvenile growth) likely have the strongest beneficial effect. We discuss the potential for both of these species to exert negative ecological effects and explore potential next steps in the risk assessment process. These examples highlight the potential utility of trait-based approaches in invasive species risk assessment and are congruent with other approaches such as the relative risk model.

**WP060 Application of a Biodiesel Co-product to Control Apple Snail: Acute Toxicity and Active Compound Identification** D. Deng, Oceanic Institute, Aquatic Feeds and Nutrition Dept; Z. Ju, W. Dominy, Oceanic Institute; A. Moriwake, E. OHara, Pacific Biodiesel, Inc.; P. Mosher, Pacific Biodiesel Technologies; P. Levin, The Hawaii Land Restoration Institute. Apple snail (*Pomacea canaliculata*) is one of the 100 worst global invaders affecting wetland crops in 18 countries. In Hawaii, the invasion of apple snails causes 18-25% annual reduction in taro crop yields and a two-fold increase in farm labor. There is still no cost effective and environmentally safe approach protecting wetland crops from this destructive pest. A previous preliminary field test showed that a biodiesel co-product, unrefined glycerin used as a soil conditioner, had caused significant mortality in apple snails. Therefore, we propose a novel approach to control apple snails by using the unrefined glycerin co-product. The objectives of this study were 1) to determine the lethal concentration (LC) of the unrefined glycerin on the



apple snail; 2) to categorize the major components of the unrefined glycerin; 3) to identify the active compound causing mortality in apple snails. The acute exposure tests were conducted in 3L beakers placed in a water bath at 25°C. There were three replications per treatment and 10 apple snails per replicate. Results of this study showed that the LC50 of the unrefined glycerin co-product for apple snail was 1.59, 0.77, 0.62 and 0.60g/L for 24, 48, 72 and 96 hours of exposure. The major compounds in the co-product used for the exposure test was methanol (27.0%), glycerin (25.3%), potassium soaps (26.0%), water (11.3%), and other salts and fatty acid methyl esters (10.7%). Potassium oleate was suggested to be a major candidate active compound in the unrefined glycerin. The pure glycerin, methanol and potassium singularly, or in combination, did not affect mortality under the test conditions used, suggesting they are not the active compounds causing mortality of apple snails. Other compounds, such as potassium soaps of different fatty acids, may also be active compounds but are not expected to be the major factor causing mortality of the apple snails. This remains to be investigated in the future. Result of this study suggested potential use of the unrefined glycerin co-product to eradicate the apple snails. Further studies, however, will be needed to test the impact of the biodiesel co-products on non-target species (other invertebrate, fish, ducks, etc.) inhabiting the same environment. Furthermore, chronic effect of the co-product will be studied in order to address issues regarding environmental sustainability and safety.

**WP061 Adaptive Management of Invasive American Bullfrogs (*Rana Catesbeiana*) in the South Okanagan Valley, Canada** N. Lukey; S. Ashpole, Univ of Waterloo; S. Murphy, Univ of Waterloo, Environment and Resource Studies; S. Murphy, Univ of Waterloo, Dept of Environment and Resource Studies. American Bullfrogs, *Rana catesbeiana*, are known to significantly and negatively impact ecosystems. Alien bullfrogs out-compete, predate upon, and transmit diseases to native amphibians. Accidentally introduced in the 1950's to the South Okanagan Valley, British Columbia, Canada, bullfrogs in recent years have been detected in 5 wetlands. We developed a three-tiered, adaptive management system in response to the bullfrog threat. The first management tier is physical removal of, and active monitoring for, bullfrogs of all stages. Removal has resulted in significant reduction in bullfrog detections from a high of 73 adults and/or juveniles in 2005 to 4 adults and/or juveniles in 2010. The second management tier assesses the risk level for future colonization or recolonization post-removal. We are using maximum entropy habitat suitability modeling in conjunction with effort analysis to determine the proportion of wetlands vulnerable to recolonization post-removal efforts, and how much human and financial resources are required for continual population suppression. The third management tier incorporates stakeholder awareness and education to facilitate monitoring of bullfrog populations. In 2010, we distributed surveys to 4 stakeholder groups to measure each group's level of bullfrog knowledge and their presence, identification, and reporting. We targeted 1) grade-school teachers, 2) landowners adjacent to at-risk or infected wetlands, 3) businesses that may act as vectors, and 4) the general public who use infected or at-risk bullfrog locations for recreation. The majority of all surveyed groups were aware of bullfrog presence, however, 90 % of landowners, 84 % of vector businesses, 50 % of teachers, and 91 % of the general public were not confident with identifying and/or reporting bullfrogs. The survey results indicate that the general public and vector businesses are greater at-risk stakeholders than teachers and landowners, and education programs need to focus on bullfrog identification and reporting avenues. Success of the stakeholder awareness and removal phases, combined with the habitat suitability model and effort analysis will ensure the long-term suppression and possible eradication of bullfrog colonies in the South Okanagan. This three tiered management system provides a strong example of a successful invasive species risk assessment and management approach to other vertebrate invasions throughout the world.

**WP062 A Risk Assessment of the Cuban Treefrog (*Osteopilus septentrionalis*) Invasion in Florida** K. Rouse Campbell, ENVIRON International Corporation, Manager, ENVIRON International Corp, Manager; T.S. Campbell, Univ of Tampa, Dept of Biology; A.J. Smith, ENVIRON International Corp. Since their initial introduction into the Florida Keys in the 1930s, Cuban treefrogs (*Osteopilus septentrionalis*) have spread rapidly throughout peninsular Florida. While Cuban treefrogs have been shown to represent a threat to native wildlife and ecosystems, few prospective assessments have been conducted on their effects on natural systems. Using the Trinational Framework for Aquatic Alien Invasive Species, data

from a long-term investigation in West-Central Florida were integrated with the literature to conduct a Cuban treefrog risk assessment. Factors evaluated to determine the probability of establishment included entry potential, colonizing potential, and spread potential, while factors assessed to evaluate the consequences of establishment included environmental impacts and risks to native treefrogs. Spatial factors that were considered in the risk assessment included the rate of spread of the Cuban treefrog invasion, how Cuban treefrogs invade new aquatic systems, projected potential Cuban treefrog distribution under future global warming scenarios, and their preferred habitats. Temporal factors included the timing of their invasion and whether tadpoles, adults, or both life stages affected native wildlife. Important life history factors considered included the rapid growth of Cuban treefrogs, the large number of eggs laid by females, the large size of females, and the fall spread of adult treefrogs to areas distant from the site of egg/tadpole development. The results of the risk assessment indicated that both the probability of establishment and the consequences of establishment of the Cuban treefrog were high.

**WP063 A Methodology to Evaluate the Economic Impacts of the Cuban Treefrog (*Osteopilus septentrionalis*) Invasion in Florida** M. McKenzie, ENVIRON International Corp.; K. Rouse Campbell, ENVIRON International Corporation, Manager, ENVIRON International Corp, Manager; G. Greene, ENVIRON International Corporation, Ecology & Sediment Mngmt. The Cuban treefrog (*Osteopilus septentrionalis*) was initially introduced into the Florida Keys and has spread northward up the Florida peninsula. Though a recent assessment has evaluated the ecological effects of this invasive treefrog on natural systems in Florida, no assessment has examined its economic impacts. Using the Trinational Framework for Aquatic Alien Invasive Species, we developed a methodology to evaluate the economic impacts of the Cuban treefrog. Three anecdotal economic effects of this species were identified. For each, a methodology was developed for attempting to collect data useful for assessing the Cuban treefrog's economic impacts. The effects examined included: 1) the impact of the species on the exotic pet trade; 2) effects on public health due to hypersensitivity reactions in humans who handle Cuban treefrogs; and 3) the impacts on power companies and utility customers associated with power outages and repairs resulting from Cuban treefrog intrusion into electric equipment. Evaluating effects of the Cuban treefrog on the exotic pet trade included contacting pet store managers and online exotic pet dealers, who were administered a set of questions regarding the sales and distribution of the animal. Evaluating the effects of the Cuban treefrogs on public health included contacting emergency rooms, county health Depts, and poison control centers in Central and South Florida to determine the incidence of reported severe allergic reactions. Lastly, a methodology was developed for acquiring data from power companies in Central and South Florida; Environmental Specialists or Operations personnel at electric power companies were contacted and questioned about power outages caused by treefrogs' intrusion into electric equipment. Preliminary results suggest ways to move forward with quantifying the most critical of economic impacts.

**WP064 Ecological and Socioeconomic Risks of Japanese Eelgrass and Its Control with Imazamox Treatment** J.P. Fisher, ENVIRON International Corporation, Principal--Sediment and Ecology Management Practice, ENVIRON International Corporation, Principal; K. Patten, Washington State Univ. While ecological functions and benefits can be prescribed to virtually all species, including non-native invasive species, the habitat and biological community changes that result from the establishment and spread of invasive species can adversely impact native species, and the socioeconomic fabric of local communities where invasions become problematic. In Washington State, Japanese eelgrass, *Zostera japonica*, introduced in the 1950's, has expanded aggressively over the past decade in coastal embayments of Washington and is expanding in portions of Puget Sound. Japanese eelgrass is not considered a noxious weed by Washington State, however, it has been associated with altered ecological functions where it has established and created conflicts in shellfish growing regions where it was not formerly present and/or prevalent. Risk assessments summarized in this presentation were conducted to consider both its ecological and socioeconomic impacts as an invasive species using Aquatic Nuisance Species Task Force guidelines, and the ecological risks from the use of the herbicide imazamox to control the species on shellfish growing areas. Adverse socioeconomic and ecological impacts to shellfish production were identified from measurements of clam recruitment and production, and benthic infauna richness on untreated

relative to treated beds. Potential effects from herbicide treatment on non-target organisms were evaluated using a food web model to explore potential exposure and effect. Based on experimental applications, imazamox appears non-persistent with a high margin of safety for asserting potential adverse effects on non-target organisms. Native eelgrass, *Zostera marina*, however, can be adversely affected from herbicide treatment, and alternative control mechanisms may need to be considered where the two species overlap. In areas where no eelgrass is found, however, Japanese eelgrass may provide habitat diversification and associated benefits. Thus, management of the non-native species represents a challenging natural resource management paradox in that protections historically afforded the species run counter to eradication and control recommendations specified under the National Invasive Species Management Plan, yet its establishment in otherwise unvegetated aquatic habitat could be interpreted to provide a surrogate for native eelgrass that has been reduced in some areas where historically present.

**WP065 Using Solid Phase Microextraction to Predict Body Residue and Toxicity of Hydrophobic Pesticides** Y. Ding, P.M. Landrum, Southern Illinois Univ; J. You, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy Sciences, State Key Laboratory of Organic Geochemistry; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology. For solid phase microextraction (SPME) to be successfully used in risk assessment, a relationship linking contaminant accumulation, toxicity in biota, and accumulation in the SPME fiber must be established. In our study, the bioaccumulation and toxicity of seven hydrophobic compounds were measured in the midge *Chironomus dilutus* larvae and amphipod *Hyalella azteca* in water-only static exposures. The seven compounds included an organochlorine pesticide dichlorodiphenyltrichloroethane (*p,p'*-DDT), and its major degradation products, dichlorodiphenyldichloroethane (*p,p'*-DDD) and dichlorodiphenyldichloroethylene (*p,p'*-DDE); an organophosphate pesticide, chlorpyrifos; and three pyrethroid insecticides, permethrin, bifenthrin, and tefluthrin. The accumulation and kinetics of the target compounds were also determined for disposable polydimethylsiloxane (PDMS) fibers. Median lethal concentration estimates based on water concentrations (LC50), measured organism concentrations (LR50), and PDMS fiber concentrations (LC50<sub>fiber</sub>) were determined for each compound and species. The relationship of total contaminant concentrations in biota ( $C_b$ ) against concentrations in PDMS fiber ( $C_f$ ) showed significant log-log linear relationships across compounds and species, regardless of whether the fiber concentration was measured after a fixed exposure time ( $C_{f4d}$ ,  $R^2=0.80$ ,  $p<0.0001$ ) or at equilibrium condition ( $C_{feq}$ ,  $R^2=0.92$ ,  $p<0.0001$ ). A significant log-log relationship was also found between  $C_{f4d}$  and  $C_{feq}$ , and  $\log C_{feq} = 0.99 \log C_{f4d} - 2.21$  ( $R^2=0.86$ ,  $p<0.0001$ ). These results demonstrated that the measured chemical concentrations in SPME fibers were correlated to the total body residue in biota and could predict exposure and therefore the toxicity of pesticides in a water medium.

**WP066 Predicting the Toxicity of Permethrin to *Daphnia magna* in Water Using SPME Fibers** A.D. Harwood, Southern Illinois Univ, Zoology, Southern Illinois Univ; A. Bunch, Southern Illinois Univ; J. You, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy Sciences, State Key Laboratory of Organic Geochemistry; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology. The multiple factors influencing bioavailability can make predictions of toxicity in natural systems difficult. The current study examined the potential use of solid phase microextraction (SPME) fibers as a matrix-independent approach to predicting the toxicity of permethrin to *Daphnia magna* across various water sources including a laboratory reconstituted water, two natural waters, and a modified natural water. Water source strongly affected the toxicity of permethrin as well as the concentration response relationships. While permethrin concentrations in the water predicted toxicity to *D. magna* for individual water sources, there was no relationship between permethrin concentrations among water sources and mortality. This indicates that differences among water sources can greatly influence toxicity, suggesting that benchmarks established using reconstituted water may be overly conservative. Additionally, while tissue residues could predict mortality for individual waters, the correlation among waters was not as clear. Finally, both 48 h and equilibrium-based SPME fiber concentrations could adequately predict toxicity independent of water properties. This demonstrates that bioavailability-based estimates provide a more accurate prediction of toxicity than water concentration and that

SPME fibers could be utilized in environmental monitoring as a rapid, accurate means of predicting toxicity in natural waters.

**WP067 Accumulation of PCBs in Passive Samplers and Biota: Field Data Analysis** K. Maenpaa, M. Leppanen, Univ of Eastern Finland, Dept of Biology; P. Mayer, Aarhus Univ, Dept of Environmental Chemistry and Microbiology; K. Figueiredo, Univ of Eastern Finland, Department of Biology; J. Akkanen, A. Kostamo, Univ of Eastern Finland, Dept of Biology; S. Herve, Finnish Environment Institute, Research and Innovation Laboratory. The scope of this study is to apply equilibrium sampling techniques to determine PCB concentrations in the aquatic environment, and to investigate the use of these methods for bioaccumulation research. The study also aims to determine the leak of PCBs downstream from the sediment of the highly contaminated lake. In 2010, polyethylene and silicone samplers were exposed for several months in the water column of the contaminated lake and along its discharge course. The samplers were measured for PCBs at several time points during the study period of six months. Sediment at each sampling site was collected and analyzed for PCBs with traditional exhaustive extraction, and also with the equilibrium passive sampling method to determine freely dissolved pore water concentrations. In addition, uncontaminated mussels were caged for two months in the water column in the contaminated lake, and native mussels also were collected from the lake sediment. Furthermore, phytoplankton was collected from the Lake water column. All of the biota samples were analysed for several PCB congeners and total lipids. The concentrations in the passive samplers were converted to lipid based concentrations and compared to the actually measured values in the organisms. The both passive sampling devices were found to be applicable to measure PCBs from the water column yielding close to similar results of the freely dissolved concentrations. Theoretical concentrations in lipids derived from passive samplers will be compared to the concentrations measured in biota.

**WP068 Bioavailable Contaminant Mixtures and Toxic Effects: Using Passive Sampling and the Embryonic Zebrafish Model to Bridge Exposure and Effects** S.E. Allan, Oregon State Univ, Environmental and Molecular Toxicology, Oregon State Univ, Graduate Researcher; B. Smith, Oregon State Univ, Environmental and Molecular Toxicology; K. Waters, Pacific Northwest National Laboratory; K.A. Anderson, Oregon State Univ, Environmental & Molecular Toxicology. The BRIDGES bioanalytical tool, which links passive sampling with the embryonic zebrafish developmental toxicity model, was developed in response to the need for methods that examine the toxicity of environmentally relevant complex mixtures. Passive sampling devices (PSDs) sequester and concentrate the freely dissolved, and therefore bioavailable fraction of hydrophobic organic contaminants, mimicking key bioconcentration mechanisms. PSD samples can be chemically analyzed as well as applied to bioassays. The embryonic zebrafish model is a widely utilized vertebrate organism bioassay. Lipid free tubing, low density polyethylene PSDs were deployed at nine different sites within, upstream and downstream of the Portland Harbor Superfund site on the Willamette River in Portland, OR for multiple 30 day periods in 2009-2010. Paired samplers were deployed at each site to obtain samples for chemical analyses and bioassays. Samples were analyzed for unsubstituted, methylated and oxygenated polycyclic aromatic hydrocarbons and screened for over 1200 chemicals of concern using Deconvolution Reporting Software (Agilent Technologies). The bioassay samples were applied to the embryonic zebrafish model. Dechorionated embryos were maintained in a static exposure solution for 5 days. Approximately 48 embryos were individually exposed to each of four concentrations of PSD extract solution from each site and sampling period. Observations of mortality and morphological endpoints were made at 1 and 5 days. Significant differences in chemical concentrations and profiles were observed between sites both within, and outside of the Superfund area. Furthermore, there were significant temporal differences, associated with seasonal changes in temperature and river flow. Significant differences in the rates of lethal and sub-lethal toxic effects were observed in zebrafish embryos exposed to PSD extracts from different sites and sampling times. Additionally, a significant dose response was observed. The BRIDGES tool proved to be sensitive to differences in toxicity between sites and times. Modeling was carried out to elucidate associations between chemicals present in the PSD samples and toxic responses observed in the exposed embryos. Suites of bioactive chemicals within the complex mixtures were identified and there was evidence of additive and synergistic toxic

effects. Furthermore, associations between specific chemicals in the mixture and developmental malformations were detected.

**WP069 Polyoxymethylene (POM) Passive Sampling Method for Measuring Pg/L Freely-Dissolved Aqueous Concentrations of Parent and Alkyl PAHs (PAH-34)** S.B. Hawthorne, Univ of North Dakota, Energy & Environmental Research Ctr; M.T. Jonker, S.A. van der Heijden, Utrecht Univ, Institute for Risk Assessment Sciences; C.B. Grabanski, Univ of North Dakota, Energy and Environmental Research Center; N.A. Azzonlina, Carnegie Mellon Univ, Civil and Environmental Engineering; D.J. Miller, University of North Dakota, Energy and Environmental Research Center. Passive sampling with non-depletive sorbents is receiving increasing interest because of its potential to measure freely-dissolved concentrations of hydrophobic organic compounds (HOCs) at very low concentrations, as well as its potential for both lab use and field deployment. However, consistent approaches have yet to be developed for the majority of HOCs of environmental and regulatory interest. In the present study, a passive sampling method was developed which allows the freely-dissolved concentrations of 18 parent and 16 groups of alkyl polycyclic aromatic hydrocarbons (PAHs) on the EPA's "PAH-34" target compound list to be measured. Commercially-available 76  $\mu\text{m}$  thick polyoxymethylene (POM) was placed in sediment/water slurries and exposed for up to 126 days, with 28 days found to be sufficient to obtain equilibrium among the sediment, water, and POM phases for the target 2- to 6-ring PAHs. In addition to measuring KPOM values for parent PAHs, since the so-called "16" alkyl PAHs on the PAH-34 list actually include several hundreds of isomers for which no standards exist, sediments impacted by coal tar, or spiked with a coal tar/petroleum non-aqueous phase liquid (NAPL) were also used to measure KPOM values for each alkyl PAH cluster. Log KPOM values ranged from ca. 3.0 to 6.2 for 2- to 6-ring parent PAHs, and correlated well with SPARC octanol/water coefficients (KOW) ( $r^2 = 0.986$ ). However, log KPOM values for alkyl PAHs deviated increasingly from SPARC log KOW values with increasing degree of alkylation. A simple empirical model that incorporates the number of carbon atoms in a PAH gave a better fit to the experimental log KPOM values, and was used to estimate log KPOM for alkyl PAHs that could not be directly measured. Detection limits (as freely-dissolved concentrations) range from ca. 1 part per trillion (ng/L) for the 2-ring PAH naphthalene, down to < 1 pg/L (part per quadrillion) for the 5- and 6-ring PAHs. Sorption isotherms were linear ( $r^2 > 0.99$ ) over at least 4 orders of magnitude. KPOM values determined independently in two laboratories (one using contaminated sediments and one using pure compounds) for several parent PAHs and PCB congeners agreed well, thus supporting the use of the commercially-available 76  $\mu\text{m}$  POM and a "standard" set of KPOM values for regulatory investigations.

**WP070 Passive Dosing Under the Microscope – Producing Gradients of Hydrophobic Organic Chemicals** D. Gilbert, S.N. Schmidt, A. Winding, P. Mayer, Aarhus Univ, National Environmental Research Institute. The environmental chemodynamics of hydrophobic organic chemicals (HOCs) are often rate limited by their diffusive mass transfer through stagnant boundary layers (SBL). This then results in concentration gradients on the micro-scale, which are crucial for both diffusive mass transfer phenomena and the chemotaxis of motile cells and organisms that are able to sense chemical signals. We present here a new experimental setup where passive dosing is employed on microscope slides, allowing for the direct visualisation of cell behaviour under tightly controlled exposure conditions. We produced microgradients of HOCs by partitioning between a loaded and clean polymer. Silicone O-rings were placed in a Dunn chemotaxis chamber whereby an outer clean ring served as a sink and a loaded inner ring as source imposing a gradient over the distance of a 1mm bridge between the rings. The gradients were well defined and remained highly stable over extended periods of time, which was confirmed by quantitative measurements. The new experimental system offers the unique possibility to study both chemotaxis of prokaryotic and eukaryotic cells exposed to gradients of chemical activity and mass transfer phenomena of HOCs.

**WP071 Incorporation of Passive Sampler and Active Biomonitoring for Assessing the Impacts of Complex Metal Pollution in Maluan Bay of China** Z. Wang, Institute of Urban Environment, Chinese Academy of Sciences, Key Laboratory of Urban Environment and Health. The development and implementation of effective remedial measures depends on our ability to predict the fate and biological effects of pollutants in aquatic

systems. To obtain a more complete picture of impacts of metal pollutants released into Maluan bay and its surrounding environments and achieve a more holistic understanding of their mechanisms on coastal marine organisms, the biomarker responses of critical tissues of indigenous clams *Ruditapes philippinarum* (digestive glands) and shrimps *Litopenaeus vannamei* Boone (hepatopancreas) were determined after 7 days in situ exposure or under laboratory conditions, which included the xenobiotic metabolizing phase II enzyme responses glutathione S-transferase (GST) and antioxidant enzymes involving in detoxifying reactive oxygen species such as superoxide dismutase, catalase and glutathione peroxidase. Moreover in order to determine the bioavailability and bioaccumulation of metal contaminants, along with active bio-monitoring of clams and shrimps, passive samplers of diffusive gradient in-thin film (DGT) were simultaneously deployed to accumulate dissolved metals in a controlled fashion, while synchronously quantifying the mean contaminant concentrations in surface water of the device, besides through the conventional methods such as measuring water quality parameters and metal contaminant levels in water columns. In this presentation, concentrations taken-up by the passive samplers will be compared to conventional methods and tissue concentrations accumulated by clams and shrimps, which showed that DGT may be able to more accurately assess the performance than other analytical techniques because of its ability to mimic the uptake of metal contaminants by biota, and innovative passive sampler technologies can be used to monitor contaminated sites and estimate metal contaminant bioavailability. Furthermore, results in this investigation also suggested that the incorporation of critical tissue residue analysis with a battery of the activity of biomarker responses in organisms after in situ cage exposure can be used as an appealing tool of active biomonitoring for assessment of metal pollution in marine coastal ecosystems. In sum, experiences from Maluan bay may be valuable for environmental management and risk assessment at contaminated sites of similar environment scenarios. (The author gratefully acknowledges the support of KC Wong Education Foundation, Hong Kong.)

**WP072 Linear Solvation Energy Relationship Models as Classifiers for the Identification of Unknowns in Environmental Samples** U. Nadin, Helmholtz Centre for Environmental Research UFZ; W. Brack, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis, UFZ Centre for Environm. Research, Dept of Chemical Ecotoxicology. The identification of unknown substances in complex environmental mixtures plays an important role in effect-directed analysis (EDA). Our approach for the identification of unknowns is based on the generation of possible structures followed by the progressive exclusion of structures that do not match experimental chromatographic and spectroscopic behaviour sufficiently. Models such as the linear solvation energy relationships (LSERs) have been applied successfully in the past to differentiate structures and retention behaviour with greater accuracy than retention indices in liquid chromatography (LC). The aim of this study is to develop a method to derive retention estimates from the proposed structures using LSERs in order to reduce the number of candidate structures in EDA. This approach focuses on the determination of the hydrophobicity index CHI using gradient elution in liquid chromatography on a capillary LC system. By prediction of CHI for candidate compounds by the LSER model and comparison to measured values of unknowns, the number of possible structures can be reduced.

**WP073 Towards a Community Driven Open Access Accurate Mass Spectral Database for the Identification of Environmental Contaminants** T. Schulze, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis; S. Emma, Helmholtz Centre for Environmental Research UFZ, Effekt-Directed Analysis, EAWAG – Swiss Federal Institute of Aquatic Science and Technology; N. Steffen, Leibniz Institute of Plant Biochemistry, Dept for Stress and Development Biology; C. Hug, C. Gallampois, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis; M. Krauss, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis, Helmholtz Centre for Environmental Research, Effect-Directed Analysis; J. Slobodnik, Environmental Institute; W. Brack, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis, UFZ Centre for Environm. Research, Dept of Chemical Ecotoxicology. A recent focus of organic environmental chemistry is nontarget screening and identification of transformation products and unknown compounds in water, soils, sediments and biological samples. Improvements in the analytical technology by accurate and multidimensional mass spectrometry (e.g., ToF-MS, QToF-MS, FT-MS, Orbitrap) in combination with liquid



chromatography and soft ionization techniques allows the analysis of a broad range of polar compounds and the restriction of the elemental composition in many cases to one or few formulae. In contrast to the large GC-EI-MS spectral databases, databases containing accurate mass spectra contain up to now relatively few spectra and are not yet widely used, as these lack many compounds relevant for environmental samples. One obstacle is the limited comparability of mass spectra generated with different MS techniques, ionization and fragmentation settings as compared to quite reproducible EI-MS spectra. However, mass spectra from different instruments share similarities if well defined experimental conditions are used for all instruments [1]. The motivation of our research is to improve the identification of unknowns in environmental samples using a common and open access mass spectral database including MS data from all instrument types and with sophisticated data evaluation tools. The web-based database MassBank (<http://www.massbank.jp>) was developed within a metabolomics consortium [2] and fulfills the prerequisites to establish a community-driven open access mass spectral database. It allows the storage of a wide variety of spectra from both GC-MS and unit and high mass resolution LC-MS<sup>n</sup>. Different tools are available to process the raw data and upload the data to MassBank including a spreadsheet based record editor for the addition of metadata. The NORMAN EMPOMASS MassBank database is hosted by UFZ and can be accessed via the NORMAN website (<http://www.norman-network.net>) or via the UFZ website (<http://www.ufz.de/index.php?en=6598>). This project is funded by NORMAN. References: [1] Oberacher, H.; Pavlic, M.; Libiseller, K.; Schubert, B.; Sulyok, M.; Schuhmacher, R.; Csaszar, E. & Köfeler, H. C. (2009): On the inter-instrument and inter-laboratory transferability of a tandem mass spectral reference library: 1. Results of an Austrian multicenter study. *J. Mass. Spec.* 44, 485-493. [2] Horai H et al. MassBank: A Public Repository for Sharing Mass Spectral Data for Life Sciences. *J. Mass. Spec.* 45, 703-714 (2010)

**WP074 Improvement of a Site Specific Screening by the Application of a Linear Solvation Energy Relationship Model** C. Hug, N. Ulrich, Helmholtz centre for environmental research – UFZ, Effect-Directed Analysis; M. Krauss, T. Schulze, Helmholtz centre for environmental research – UFZ; W. Brack, Helmholtz Centre for Environmental Research UFZ, Effect-Directed Analysis, UFZ Centre for Environm. Research, Dept of Chemical Ecotoxicology. For the assessment of waste water treatment plant (WWTP) effluents and their possible impact on benthic organisms in receiving waters, target analysis of a limited number of the most frequently identified compounds in treated waste water and surface water is not sufficient. The investigated WWTP handles mainly sewage from chemical industry facilities and minor shares of municipal waste water and groundwater contaminated by former industrial activities. We compiled a site-specific suspect list of more than 1800 organic chemicals produced in this area. Solid phase extracts of treated sewage were analyzed by a liquid chromatography-high resolution mass spectrometry using a LTQ Orbitrap XL system. Exact monoisotopic masses of suspects were automatically searched by the MZmine software in LC-MS chromatograms in different ionization modes. Chromatographic retention time of the suspects whose exact masses were present as a peak in chromatogram were predicted from structure by a Linear Solvation Energy Relationship (LSER) model. The LSER model was adapted to applied chromatographic conditions. Based on a prognosis interval of 2.6 min in a 21 min gradient, two-thirds of the 300 suspects with present mass were excluded. MS/MS spectra were acquired for the remaining suspects showing peaks fitting to the predicted retention time. MS/MS fragments were compared with data from literature and MS/MS databases. About twenty compounds produced at the site were tentatively identified. A final identification was performed by commercially obtained reference standards. The application of the LSER model improved the exclusion of suspects compared to previous used correlations of parameters as log  $K_{ow}$  value and retention time. By the performed data processing several side specific pollutants were identified in an accelerate way.

**WP075 Identification of Thyroid Hormone-like Compounds in Polar Bear Plasma by Effect-Directed Analysis** E. Simon, BioDetection Systems; J. Bytingsvik, Norwegian Univ of Science and Technology, Dept of Biology; T. Hamers, P. Leonards, J.d. Boer, Institute for Environmental Studies, VU Univ; B.M. Jenssen, Norwegian Univ of Science and Technology, Dept of Biology; J. Aars, Norwegian Polar Institute; E. Lie, The Norwegian School of Veterinary Science, Dept of Food Safety and Infection Biology; M. Lamoree, Institute for Environmental Studies, VU Univ,

Chemistry & Biology, Institute for Environmental Studies, Chemistry & Biology. Endocrine disrupting compounds (EDCs) released into the environment may bioaccumulate and biomagnify in the foodchain. Significant concentrations of thyroid hormone-like (TH-like) compounds were found in biological tissue (e.g., in fat and blood) of top predators such as humans and polar bears (*Ursus maritimus*). This study aims to identify TH-like compounds in plasma of polar bear cubs-of-the-year by Effect-Directed Analysis (EDA). Solid-phase extraction (SPE) followed by liquid-liquid extraction (LLE) was evaluated for known TH-like compounds (hydroxylated polychlorinated biphenyls (OH-PCBs), hydroxylated polybrominated diphenyl ethers (OH-PBDEs), other halogenated phenols (OHPs) and perfluorinated compounds (PFCs)) as well as the less potent non-hydroxylated parent compounds of the PCBs and PBDEs spiked to cow plasma. Good chemically determined recoveries were obtained for the hydroxylated compounds extracted from spiked plasma (>90%) and lower recoveries for PFCs (~60%) and for PCBs and PBDEs (~30%). In the radioligand T4\*-TTR binding assay the extracts showed TTR binding potencies which were in good agreement with the calculated theoretical spiking levels. Using the validated method, the polar bear plasma samples were extracted and screened in the T4\*-TTR binding assay. Measured TH-like activities were generally higher than the estimated activity based on the measured concentrations of target TH-like compounds. Three samples were selected for further analysis, where the analyzed target compounds could only explain part of the measured TH-like activity, to identify the compounds causing the remaining activity. The selected extracts were analyzed by GC-MS operated in full scan mode and showed relatively simple chromatograms. Therefore, the identification of unknowns causing the unexplained TH-like activity was conducted by accurate mass measurements on GC- and LC-TOF (Time-of-Flight)-MS without further fractionation. In addition, GC-ECNI-MS was carried out to identify chlorinated and brominated known TH-like compounds. The presence of OH-PCBs in the polar bear plasma extracts could be confirmed, as well as the presence of the compounds that had been tentatively identified using the GC-MS screening method. Currently, testing of the biological activity of the analytically confirmed compounds is underway and more in-depth identification of unknown toxicants is scheduled, using high resolution mass spectrometry in combination with both liquid and gas chromatography.

**WP076 The Potential of Selective Combinations of Strains of Salmonella to Enhance Mutagenicity Characterization of Air Samples and Guide Chemical Analysis** D.K. Alves, USP, FCF; D.A. Morales, UNICAMP, Leal, FT; A.A. Albuquerque, UNICAMP, FT; F. Kummrow, UNIFESP; G.A. Umbuzeiro, UNICAMP, FT, UNICAMP, Professor. Atmospheric particulate matter is recognized as mutagenic and although several compounds have been identified in this matrix, they do not seem to explain the effect observed when the same sample is tested in bioassays. The Salmonella/microsome mutagenicity assay has been largely used for the evaluation of atmospheric samples. It is very sensitive can detect various types of mutagens because it is performed with different strains with and without exogenous metabolic activation (S9). The objective of this work was to verify the applicability of selective pairs of strains of the Salmonella/microsome assay to characterize the mutagenicity of airborne particulate matter collected in a city in the country side of São Paulo State, Limeira, known to be highly polluted because of intense traffic, industrial and agricultural emissions including from sugarcane plantations. We tested six extracts obtained with methylene chloride by ultrasonication using the microsuspension method in the presence and absence of rat liver S9 in dose response experiments, with maximum doses of 50 µg of extractable organic matter (EOM) per plate. The following pairs of strains were used: YG1041/TA98, to verify the presence of nitro-compounds, as 1-nitropyrene and/or aromatic amines, YG5161/TA1538, highly sensitive to non-substituted polycyclic aromatic hydrocarbons (PAHs) as benzo[*a*]pyrene (BaP) and YG7108/TA1535, for the indication of alkylating agents, as ethyl methanesulfonate. The results indicated that the compounds that could explain better the observed effect are the nitro/aromatic amines and not non-substituted PAHs such as BaP. The pair YG7108/TA1535 provided negative responses for all the samples. The comparison of the observed responses for the samples analyzed the mutagenicity profiles of pure compounds found in the literature did not provide any candidates that could alone represent the mixture. More mutagenicity studies with pure compounds using the proposed pair of strains along with chemical analysis of fractionated extracts are needed to verify if this approach can be useful in EDA (effect directed analysis) studies and consequently in

the discovery of new air priority pollutants. Acknowledgements: The authors thank CNPq and Capes.

**WP077 Effects-Driven Fractionation of Heavy Fuel Oil to Isolate Compounds Toxic to Trout Embryos** J. Bornstein, Queen's Univ, Chemistry, Queen's Univ, Chemistry Dept; J. Adams; B. Hollebone, Environment Canada, Emergencies Science & Technology; P.V. Hodson, Queen's Univ, School of Environmental Studies; R.S. Brown. Recent oil spills have raised many issues about potential harm to aquatic and marine life. For risk assessment, an important question to ask is, "Which compounds in the oil have the potential to cause the most damage, due to both bioavailability and toxicity?" Heavy fuel oils used by large cargo ships and cruise liners are quite thick and viscous, and contain a variety of high molecular weight hydrocarbons, many of which cause acute and chronic toxicity to fish. Due to the high frequency of small volume transports, the risk of a spill of heavy fuel oils is quite high. Thus, it is critical to assess the toxic properties of such oils, and to compare them with lighter oils that might be better characterized. A multi-disciplinary approach was used to identify the oil components that are most toxic to the early life stages of rainbow trout. Bunker C (a Heavy Fuel Oil) was fractionated to identify which of its chemical constituents are most closely associated with toxicity to embryonic fish. First, a vacuum distillation apparatus was used to divide the oil into four fractions by boiling point. Based on toxicity testing, fraction F3, the most toxic fraction, and the one containing most polycyclic aromatic hydrocarbons (PAHs), was further separated into fractions rich in asphaltenes, waxes, and PAH, according to their relative solubility in cold solvents (e.g., acetone). The wax precipitation was optimized for solvent, solvent-to-oil ratio, and precipitation temperature. Finally, the PAH-rich fraction (F3-1) was separated by HPLC into sub-fractions by the approximate number of aromatic rings per molecule. For each fraction, GC-MS, GC-FID and fluorescence were used in addition to HPLC to measure PAH and alkyl-PAH concentrations, and toxicity tests with rainbow trout identified which fractions and groups of compounds were associated with acute and chronic toxicity, recognizing physical and chemical properties such as size, shape and Log  $K_{OW}$ .

**WP078 Advances in Ecological Modeling for Assessing Ecological Risks and Ecosystem Services** M. Convertino, Univ of Florida, Agricultural and Biological Engineering; G. Kiker, Univ of Florida; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center. The anthropogenic pressure and climate change often result in species shifts that are broadly categorized as: decline, extirpation, extinction, and population boom. Early warning of population shifts is necessary for efficient environmental management, including adaptive ecosystem management in which monitoring plays a big role. We propose a new analytical model for predicting shifts of species coupling space-time patterns. A model based on a 2-D lattice for a bird guild tested the theory here introduced. The probabilistic risk model uses the abundance of the species considered that is one of the outputs of common metapopulation models. Early-warning classical signals of shifts are the variance and the autocorrelation of the abundance of a particular species. The fractal dimension was introduced as a more meaningful early-warning signal combining the autocorrelation and the variance of the abundance in space and time. In order to capture the shift the fractal dimension has been monitored in time for minimum 3 species generations. Shifts produce change in the spatial configuration of species, e.g., transitions from random to clustered patterns and viceversa. The species risk at-a-point has been defined as the expected probability of shift occurrence (hazard) of a given intensity which takes place in a certain area within the landscape at a given time. We calculate the total risk for all the predicted shift occurrence (exposure), that is thereby representative of the sum of all the risk at-a-point. We assume a power-law model of damage for the ecosystem (vulnerability) that is a function of the lost/gained species abundance and the species-dependent threshold of decline and extinction. The model is promising for testing the response of different species under varying degrees and types of exogenous stress (e.g., climatic, toxicological) and anthropic interventions possibly aiding decision-making for ecosystem adaptive management policies.

**WP079 An Individual-Based Model for Extrapolating Standard Toxicity Test Data into Population-Level Adverse Outcomes in the Fathead Minnow** A. Olmstead, US Environmental Protection Agency, Mid-Continent Ecology Division; M. Etterson, R. Bennett, D. Hoff, J. Hoffman, R. Johnson, P. Schmieder, US Environmental Protection Agency. While

environmental toxicity testing typically focuses on organism-level endpoints such as mortality, growth, and reproduction, risk assessment guidelines specify protection goals at the level of the population and above. One method of linking these different levels of biological organization is the development of models that extrapolate toxicant impacts at the individual level to adverse outcomes at the population level. However, annual population growth projections in highly fecund species such as fathead minnow are subject to extreme uncertainty. We have developed an individual based model for the fathead minnow that integrates adverse effects of stressors on individual growth, reproduction, and survival over a single breeding season using toxicity data inputs from standard toxicity tests such as the early life stage and full life cycle assay. The model uses a daily time step and projects the size-distribution of the population at freeze-up, which is a reliable indicator of the over-winter potential of a fathead minnow population. Specific model outputs are population biomass, census, and size structure. Exposure inputs allow for varying the timing of the exposure in order to compare the effects of various temporal patterns of contamination. Advantages of this type of modeling framework are the ability to introduce greater realism, including density-dependent factors. Data outputs are amenable to long-term, multi-year population modeling if sufficient data on overwinter survival exists.

**WP080 Improving Population Models for Assessing Pesticide Risks to Pacific Salmon** R. Pastorok, B. Parks, D. Preziosi, Integral Consulting, Inc. Under Endangered Species Act (ESA) consultations supporting Federal Insecticide Fungicide and Rodenticide Act assessments, the National Marine Fisheries Service (NMFS) issued several biological opinions (BiOps; 2008-2010) that evaluate risks of certain organophosphate (OP) and carbamate pesticides to Pacific salmon. Population modeling was one approach NMFS used to evaluate the potential effects of pesticides on salmon. We present a critique of these population models and their application in the BiOp for OPs to illustrate problems that may arise when applying such models in an environmental regulatory context. NMFS developed density-independent population matrix models to estimate changes in population growth rate for four salmon life-history types. In each case, the population matrix model was linked to toxicity models to separately evaluate: 1) changes in subyearling salmon growth and survival resulting from decreases in feeding rate (via pesticide inhibition of brain acetylcholinesterase activity) and reduced prey abundance and 2) acute lethality of pesticides to subyearling salmon. Although linking population projection models to sublethal and lethal toxicity models can enhance a risk assessment, our evaluation of these models revealed critical shortcomings for their intended application. Essentially, they were screening-level analyses which are insufficient for a determination of jeopardy under the ESA. For example, assumptions of density independent population growth and no spatial structure for the salmon populations ignore basic biological/ecological processes known to be important in Pacific salmon populations. Exposure assumptions used in the BiOps were invalid, including: 1) all salmon are exposed within a water body where pesticides are applied in the watershed, 2) all salmon subyearlings use off-channel habitats with still water, and 3) pesticide exposure concentration is constant over the exposure and orders of magnitude above what has been found in salmon streams in the Pacific Northwest. Data inputs for the toxicity models were also overly conservative. We recommend a strategy for developing improved models to provide more realistic spatially-explicit assessments of exposure, toxicity, and risk to support population-level risk analysis for Pacific salmon.

**WP081 NOAA HEA Tools: A Spatially Explicit Framework for Habitat Equivalency Analysis** B. Shorr, NOAA/Orr; D. Hudgens, A. Geel, Industrial Economics; Z. Nixon, Research Planning Inc.; A. Dvorskas, T. Penn, NOAA/Orr; R. Neely, NOAA. Habitat Equivalency Analysis (HEA) has become an industry-standard analytical approach in natural resource damage assessment to quantify ecological injuries and scale compensatory restoration actions. Over time, the method has been increasingly applied to more complex sites, including sites with multiple habitat types, sites characterized by multiple contaminants at varying concentrations, and sites subject to different types of primary remediation at various points in time. Performing HEA calculations that take into account all these factors, particularly when combined with the desire for a higher degree of spatial resolution and the ability to evaluate a variety of scenarios, requires a great deal of model complexity and computational power. Performing and effectively communicating these analyses also require more flexibility than can be accommodated by the spreadsheet tools most commonly used to perform HEA calculations. NOAA's HEA Tools include toolboxes built in ESRI® ArcGIS software, and

a database calculation application built in Microsoft® Access. These flexible and scalable tools facilitate geospatially explicit calculations of estimated injuries. The tools are designed to provide functionality for simple (single contaminant) to complex (multiple contaminant) sites and scenarios. The tools allow users to enter multiple HEA inputs as distinct “scenarios” to enable sensitivity analyses. The tools also enable users to input characteristics of compensatory restoration projects and estimate the scale necessary to compensate for site injuries. A key feature behind this approach is the custom application that allows the user to access HEA functionality from a coordinated navigation system. The framework in both the GIS environment and guided navigation forms in MS Access helps guide the scientific team through the collection and processing of data, spatial analyses, and HEA calculations. The tools also automate generation of metadata and processing steps and outputs of spreadsheet and GIS data layers to facilitate communication and negotiation of damage assessment.

**WP082 A Simple Ecosystem Model to Predict Endocrine Disruptions in the Aquatic Environment** L. Clouzet, Université Laval; M. Paterson, D. Findlay, P. Blanchfield, K. Mills, Fisheries and Oceans Canada; K. Kidd, Univ of New Brunswick; P. Vanrolleghem, Université Laval. Ecological risk assessment (ERA) requires simple tools to predict critical changes in ecosystems. Nowadays, ERA is evolving from individual measurements to population modeling. However, a chemical's impact needs to be evaluated at higher levels of organization to guarantee reliable ERA. Ecosystem models are considered too complex and too specific to be applied in ERA. This study therefore aims to provide a simple ecosystem model to risk managers to support the Ecosystem Services concept. Considering the increasing concern towards endocrine disruption in aquatic ecosystems, an additional objective is to add appropriate reproductive endpoints. The model was developed to consider direct effects of chemicals but also ecological interactions (feeding and competition relationships). In the software package WEST, an object-oriented model was built with different trophic levels: phytoplankton, zooplankton, planktivore and piscivore fish. Equations from the AQUATOX model (USEPA, 2002) were simplified and adapted to describe endocrine disruptions. The model consists of (i) a food web model (ii) toxic effect sub-models and (iii) a model for nutrient and detritus cycling. The model was developed with data obtained from a unique experimental lake study. A multi-year whole-ecosystem study was performed at the Experimental Lake Area (ELA, Ontario, Canada) with exposure of well-defined fish and lower-trophic-level populations to environmentally-relevant concentrations of the synthetic hormone 17 $\alpha$ -ethinylestradiol (EE2). The experimental lake study was divided in three steps (i) baseline data (ii) EE2 addition and (iii) recovery data. The fathead minnow population collapsed after the second year of EE2 addition and endocrine disruption was observed in other species (Pearl dace, Lake trout, White sucker). Direct effects of EE2 were concluded from this experimental lake study and hypotheses on indirect effects such as decrease of some species were raised. The model was developed with the baseline data, calibrated with the data collected during EE2 addition and validated with the recovery phase. To assess the reliability of this simple ecosystem model, other experimental lake studies in the ELA will be modeled.

**WP083 Aquatic Risk Assessment of Chemicals Supported by Individual and Population Level Models – A Contribution of the CREAM Project** U. Hommen, Fraunhofer IME. In September 2009, CREAM, a Marie Curie Initial Training Network funded by the European Commission, was initiated. Currently, 20 PhD students and 3 postdocs are employed at the 13 host institutions to develop ecological models for the risk assessment of chemicals, primarily pesticides. What is unique and important is that this network prioritizes also the involvement of 10 associated partners from industry, regulatory authorities, and contract research organizations, in order to efficiently tailor the results of the projects to the actual risk assessment needs in practice. Several of the projects deal with aquatic populations, i.e., invertebrates and fish, and aim to contribute to questions such as: How can we predict toxicity on the organism level, e.g., for different exposure patterns? How can we estimate the consequences of lethal and sublethal organism level effects on the population level? How can we extrapolate effects and recovery from local to regional scale? The adopted approaches range from laboratory experiments and trait analyses, to toxicokinetic/toxicodynamic models, matrix population and individual-based models, as well as geographically referenced fate-and-effect modelling on a landscape-scale. The poster will shortly describe each of the projects on aquatic organisms, including the main results achieved so far.

**WP084 From Laboratory Derived Individual Level Endpoints to Population Level Risk Assessment** C.R. Hazlerigg, Imperial College, Division of Biology; K. Lorenzen, Univ of Florida, School of forest resources and conservation; C. Tyler, Univ of Exeter; J. Wheeler, Syngenta Ltd, Environmental Safety; P. Thorbek, Syngenta Ltd. Most ecotoxicological endpoints used in risk assessments are measures taken at the level of individuals and derived from laboratory tests. For fish, typical endpoints are mortality, growth, development and sometimes reproduction. However, protection goals for wildlife are generally at the level of the population. Population level processes such as density dependence and behavioural interactions are also important, meaning that translating simple effect measures from laboratory based studies directly to population level effects is extremely difficult. To investigate how toxic effects and population level processes may interact, we have formulated an individual-based zebrafish population model. The zebrafish was chosen as the model species due to its use in laboratory ecotoxicity tests for risk assessment. The model was designed and parameterised based on results from a survey of wild fish, semi-field experiments and data derived from an extensive literature review. The model, written in NetLogo (CCL, Northwestern Univ), mimics a 6m<sup>2</sup> pond with a habitat appropriate to zebrafish found in the wild. Zebrafish development was divided into four life-stages (eggs, larvae, juveniles and adults) with life history characteristics adjusted with each time-step. The model was used to measure the sensitivity of the population to changes in those individual-level endpoints used in OECD laboratory tests (e.g., hatch success, growth). These individual-level processes were decreased by 10, 20 and 50% and the resulting changes to population abundance measuring the sensitivity of the population response. Each simulation ran for 1500 days and each scenario was repeated 70 times. Different individual-level endpoints had very different response sensitivities in the model. Whilst some endpoints, including growth, showed an immediate effect at the population level, others had limited effects, especially at lower levels of disruption (e.g., sex ratio). The compensatory processes in the population (density dependence) appeared to absorb these effects so that limited or no population-level responses were identified. We rank the tested individual-level endpoints in order of their effect at the population-level. The goal of this study was firstly, to demonstrate how ecological modelling can aid in extrapolating from individual-level endpoints to population-level endpoints and secondly, how these models can help prioritise which endpoints are most important at the population level.

**WP085 How Can Ecology Help Us Predict the Effects of Contaminants on Population Dynamics?** A. Palmqvist, Roskilde Univ, Environmental, Social and Spatial Change, Roskilde Univ, Dept of Environmental, Social & Spatial Change; V. Forbes, Univ of Nebraska Lincoln, School of Biological Sciences. Marine deposit-feeding polychaetes belonging to the *Capitella* sibling species complex are classic re-colonizing species frequently used as an indicator of habitat disturbance (natural and anthropogenic). Within this species complex some species are very opportunistic whereas others are both less mobile and less tolerant to disturbance. Some of these sibling species have been extensively used for both laboratory experiments and field surveys, though in the field different sibling species are indistinguishable based on morphological characteristics alone. With a combination of various types of laboratory experiments and model simulations the present study aims to pinpoint the most important factors driving the dynamics of field populations of *Capitella* spp., in order to focus further research, monitoring, and management of disturbed areas. The importance of larval dispersal ability and settling behaviour was explored through both a matrix metapopulation study and targeted settling experiments. With increased focus on incorporating more ecological relevance into ecotoxicological studies, we designed experiments to mimic and explore the boom-bust dynamics of *Capitella teleta*. Our results emphasize the influence of dispersal ability and settling behavior on the population dynamics of re-colonizing polychaetes and highlight the importance of understanding the principal factors driving re-colonization of disturbed sediments. Not surprisingly, food availability seems to be the main driver for both larval dispersal and settling behavior as well as the main determinant of density dependence in *Capitella* spp., and is more important for settling in organically contaminated areas than is the presence of sediment-associated contaminants at ecologically relevant concentrations. The take-home message from this study is that in order to extrapolate to field populations for risk assessment and management purposes, it is necessary to choose representative model species for which we understand and can incorporate key aspects of their ecology and biology. Such knowledge can



help to focus testing and research on endpoints that are more relevant for predicting effects on populations.

**WP086 How Does *Hyaella azteca* Respond to Pulse Exposure of Environmentally Realistic Concentrations of Permethrin?** S. Pedersen, Roskilde Univ, Environmental, Social and Spatial Change, Roskilde Univ, Dept of Environmental, Social and Spatial Change; A. Palmqvist, Roskilde Univ, Environmental, Social and Spatial Change, Roskilde Univ, Dept of Environmental, Social & Spatial Change; P. Thorbek, Syngenta Ltd, Environmental Safety, Syngenta, Environmental Safety; M. Hamer, Syngenta Ltd; V. Forbes, Univ of Nebraska Lincoln, School of Biological Sciences. Exposure of non-target aquatic organisms to pesticides often occurs in short pulses following periods of drain flow, surface run-off or spray drift. Since standard aquatic toxicity tests for acute effects assessment are primarily based on continuous and maintained exposure periods on individuals, there is a mismatch between laboratory and field exposure patterns. The aim of the present study was to examine the potential for short-term and delayed effects of different pulse exposures and concentrations of a pyrethroid pesticide, permethrin, on the freshwater amphipod *Hyaella azteca*. Two different tests with juveniles of *H. azteca* were conducted to test the effect of pulse exposure. In the first test, *H. azteca* were pulse exposed to a single pulse of different nominal concentrations of permethrin; 0, 0.1, 0.3 and 2.7 µg/L. The exposure pulse lasted for either 1 or 3 hours. In the second test, *H. azteca* were exposed to two repeated pulses of one hour each with different intervals between the two pulses. The nominal exposure concentrations were 0, 0.3 or 0.9 µg/L, and the intervals between the pulses were 0, 6, 24, 48 or 144 hours. After a pulse the organisms were transferred to clean water. Mobility was recorded immediately after a pulse and again after 10 days from the start of the experiment and continuously until the end of the experiment. Each week the beakers were also monitored for juveniles. Pulse exposure of permethrin significantly affected mobility of *H. azteca*. Some immobile individuals regained mobility after transfer to clean water and there was no indication of delayed effects on mobility. The results suggest that exposure concentration has a higher impact on mobility than pulse duration. Mobility, time to first reproduction, and reproductive output were not affected by the interval between the two pulses for the tested concentrations. There were no marked differences in mobility after the first pulse and the second pulse. The data will eventually be integrated into an individual-based model to provide information on how a population of *H. azteca* will respond after exposure to pesticide and the potential for the population to recover. This approach will be more ecologically relevant compared to the normally used constant exposure and can be used as a tool to provide information regarding ecological risk.

**WP087 How Much of a Difference in Assessing Ecological Risk Between Effects of Organism-Level and Population-Level?** B. Lin, National Institute of Advanced Industrial Science, Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, Research Institute of Science for Safety and Sustainability. Most protection goals stated in environmental regulations or policies are aimed at the population level or higher and a movement toward population-level ecological risk assessment (ERA) is gaining acceptance for support of chemical management in recent years. However, due to the reason that population-level ERA is a data-intensive analysis (it requires much more ecotoxicity data than organism-level), assessing the ecological risk of toxic chemical is mainly based on a limited number of measures of organism-level effects in laboratory toxicity tests. Thereby, there is an enormous concern in understanding how much of the difference in assessing the ecological risk between effects of organism-level and population-level. To get a rough answer to this concern, this study performs a comparative study on the difference between the derived reference values (PNECs) for risk calculation from approaches of organism-level and population-level. To derive the reference value defined as  $C_{\lambda=1}$  for use in population-level ERA, extrapolation approach (Lin and Meng, 2009) using available acute ( $LC_{50}$  or  $EC_{50}$ ) and chronic (NOEC) toxicity test data is employed. From the comparative results of selected chemicals, a range of difference will be shown and discussed. This study is expected to be help in bridging the gap between the protection goals and practical ecological risk assessment. Lin and Meng (2009) "Extrapolation of Available Acute and Chronic Toxicity Test Data to Population-Level Effects for Ecological Risk Management of Chemicals," *Environmental Toxicology and Chemistry*, 28(7): 1557–1566.

**WP088 Matrix Modelling to Compare Population Sustainability of Realistic Worst-case Fish Species for Pesticide Risk Assessment in the EU** L. Ibrahim, Fraunhofer IME; T.G. Preuss, RWTH Aachen Univ, Institute for Environmental Research; U. Hommen, Fraunhofer IME. The assessment of potential effects of chemicals, e.g., pesticides, on fish in Europe is usually based on ecotoxicological tests, taking non-European species as surrogates. These tests deliver organism-level endpoints (e.g., effects on survival, growth and reproduction). However, because the goal is to protect individuals and populations of species native in Europe, there are two types of extrapolation which might improve risk assessment for fish: from test species to ecologically relevant species and from (sub-)lethal effects on individuals to the population level. Hence, we intend to develop a population model of a realistic worst case fish species to support pesticide risk assessment in the EU. For this purpose, first a list of 11 most potentially vulnerable species was concluded via a step-wise screening of a comprehensive list of European freshwater fish based on their potential exposure to pesticides. These 11 species are native to Europe, widespread in the EU (at least in one of the three zones of mutual recognition for pesticide regulation) and are reported in and dependent on edge-of-field water bodies. Some of them are protected under the EU habitats directive. Then, these species were ranked for population sustainability based on their life history traits; matrix modeling was used to analyze the relative effects of reduced development, survival and/or reproduction rates on the population growth rate of the different species. Consequently, based on these results as well as the availability of additional information on the species, including field data for model testing, one species will be chosen for a more detailed analysis by means of an individual-based population model which will be developed.

**WP089 Population Level Risk Assessments – Science or Fiction?** M. Wang, Rifcon GmbH, Dept Efat & Modelling. Population models are considered as a rather novel method for risk assessment on the population level. Such models have a long history in ecology and they are increasingly being used also for the risk assessment of pesticides. However, it is frequently debated to which extent population models are useful for risk assessment and if their output can be considered being realistic or if they produce rather fictitious predictions. We address this question by analyzing different published population models developed specifically for pesticide risk assessment. We demonstrate advantages and disadvantages of using population models in risk assessment compared to established assessment methods, especially when addressing protection goals within the ecosystem services concept.

**WP090 The Use of Bayesian Networks to Integrate Population Modeling, Community Interactions and Ecosystem Services** W.G. Landis, Institute of Environmental Toxicology, Western Washington Univ, Western Washington Univ, Institute of Environmental Toxicology, Western Washington Univ, Institute of Environmental Tox. & Chem.; K.K. Ayre, Western Washington Univ, Institute of Environmental Toxicology. One of the difficulties in environmental toxicology and risk assessment has been in creating a framework where toxicants, other stressors, populations, community interactions, landscape features and ecosystem services can be combined in a predictive manner. Bayesian networks can be used as such a tool to tie the effects of stressors to large scale ecological functions such as habitat quality, water condition and landscape characteristics to predict risk to ecological services. This use of Bayesian networks has already been demonstrated in the case of whirling disease affecting valuable populations of cutthroat trout in the American Southwest. This study incorporated habitat for the pathogen, its intermediate host and the trout and the distribution of that habitat into a risk of infection. Also it is also possible to incorporate the output of probabilistic population models such as produced by RAMAS or other tools into a Bayesian network via the conditional probability tables (CPTs). The CPTs inform the interactions between parent and daughter nodes of the Bayesian network. One of the characteristics of Bayesian networks also is the ability to take specifications for ecosystem services and being able to back calculate to the appropriate initial conditions, or management activities, to produce the desired outcomes. The nature of the BN approach is that it is also very clear what critical data are missing in order to produce predictions with acceptable uncertainty. We will present case studies, such as the Pacific herring of Cherry Point WA, of using BNs in conjunction with population models and field data to model large-scale ecological functions and relate them directly to ecosystem services. In conclusion, it is very possible to combine population models, cause-effect interactions and ecological interactions into probabilistic models of risk to ecosystem services.

**WP091 Use of the Comprehensive Aquatic Systems Model for Atrazine (CASMATZ) to Estimate Potential Changes in Primary Producer Community Structure** R. Brain, Syngenta Crop Protection, LLC., Dept of Environmental Risk Characterization; P. Hendley, Syngenta Crop Protection, LLC., Senior Syngenta Fellow; S.B. Wall, Syngenta Crop Protection, LLC., Ecological Sciences; A. Hosmer, Syngenta Crop Protection, LLC.; S. Bartell, E2 Consulting Engineers, Inc. In 2003, the USEPA required an intensive stream water exposure monitoring program to determine the potential impact of atrazine on the aquatic primary producer community structure. The Comprehensive Aquatic Systems Model (CASM) was parameterized to use atrazine laboratory toxicity data and region-specific characteristics to translate daily atrazine exposure concentrations generated from the stream monitoring program into estimated potential effects on individual model populations of aquatic primary producers and consumers in a generalized 2<sup>nd</sup>–3<sup>rd</sup> order Midwestern US stream. CASM\_Atrazine (CASMatz) is a bioenergetics-based approach that describes daily production dynamics for a user-specified aquatic food web. It considers the combined implications of varying exposure concentrations/durations, changing environmental conditions, differential population sensitivity to atrazine, and grazing/predator-prey relations in estimating any potential direct and indirect risks posed by site-specific measured atrazine chemographs. The generic stream producer community consists of populations of phytoplankton, periphyton, and macrophytes while populations of zooplankton, benthic invertebrates, decomposers, and fish constitute the consumers. In reference simulations, individual population biomass (carbon) values vary as nonlinear functions of daily values of light, temperature, nutrients (N, P, Si), current velocity, water depth, and complex grazing and predator-prey interactions. CASMatz estimates of magnitudes and temporal patterns of population biomass in the absence of other stressors are similar to those reported for 2<sup>nd</sup>/3<sup>rd</sup>-order streams. CASMatz provides the flexibility to use alternative methods to estimate exposure-response based on an extensive database of atrazine laboratory toxicity studies and the resulting modelled changes in producer population biomass for varying atrazine exposure scenarios compare favorably with effect and recovery results measured in 33 atrazine microcosm/mesocosm experiments. This generic stream version of CASMatz demonstrates that potential population-level responses to time-varying herbicide chemographs can be characterized for risk assessment using bioenergetics-based models. Importantly, CASMatz provides ecological complexity commensurate with more realistic modeling of potential changes in aquatic primary producer community structure and consumer communities.

**WP092 Using Ecological Models in Chemical Risk Assessments of Vertebrates: Contributions from CREAM** P. Thorbek, Syngenta Ltd, Environmental Safety, Syngenta, Environmental Safety. Mechanistic Effect Models for Ecological Risk Assessment of Chemicals (CREAM) is a Marie Curie Initial Training Network, funded by the European Commission within the 7th Framework Programme. CREAM includes 20 PhD and 3 postdoc projects. All projects are related to developing ecological models for the risk assessment of chemicals. CREAM has full and associated partners from industry, regulatory authorities and contract research organizations. The Vertebrates Work Package consists of 8 projects covering both aquatic and terrestrial habitats and chemicals ranging from metals over PCBs to pesticides. The following organisms are being modelled: wood mouse, bats, skylark, wood pigeon, polar bear and fish. The model types range from toxicokinetic models, over foodweb bioaccumulation models and matrix models to individual based models. Some models predict field exposure and the toxic effects this might cause; others predict the population level effects of both chronic and acute toxic effects. Here, the different projects and the main results produced so far will be shortly presented. The Vertebrate Work Package includes the following fellows: Agnieszka Bednarska (Applying toxicokinetic modelling to wildlife risk assessment for pesticides), Chun Liu (Modelling the importance of exposure patterns, life history traits & toxicokinetics for the risk to populations of small mammals), Beatrice Hernout (Modelling the effects of soil contaminants on the bat population in the UK), Tomasz Kulakowski (Ecological modelling to assess the risks that pesticides pose to skylark populations), Katarzyna Matuszewska (Ecological modelling to assess the risks that pesticides pose to woodpigeon populations), Viola Pavlova (Modelling effects of chemicals on polar bear population dynamics), Julita Stadnicka (Predicting toxicity to fish based on in vitro data via a two step model) and Lara Ibrahim (Towards a more realistic chemical risk assessment for fish: development and use of population models).

**WP093 Using Population Models to Guide Management Activities: Optimal PCB Remediation Strategies Based on an Individual Based Model of Mink** C.J. Salice, Texas Tech Univ/TIEHH, Environmental Toxicology, Assistant Professor, Texas Tech Univ; B. Sample, Ecological Risk, Inc.; R. Miller Neilan, Louisiana State Univ, Dept of Oceanography and Coastal Sciences; K.A. Rose, Louisiana State Univ, Oceanography and Coastal Sciences; S. Sable, Louisiana State Univ, Dept of Oceanography and Coastal Sciences. The use of population models in ecotoxicology and ecological risk assessment has increased in recent years. A key advantage is that population models can be used to place observed toxic effects into an assessment of the impacts on population-level endpoints, which are generally considered to provide greater ecological and societal relevance. The most common applications of population models have been to assess potential effects of contaminants. An additional use is in evaluating the potential outcomes of various remediation strategies for particular populations of concern. We used an individual-based model of mink to evaluate the population-level effects of exposure to polychlorinated biphenyls (PCBs) and the impact that different remediation strategies had on mink population endpoints (population size and extinction risk). An individual-based approach was chosen because it allowed us to explore the population-level consequences of individual variation in PCB exposure as may occur in habitats with heterogeneous distributions of contaminants. The model was parameterized with data from previously published studies and reports and included both stochasticity and density dependence. Although the model was not spatially explicit, *per se*, we created certain “hot spots” of contamination within the mink population to approximate a spatially explicit approach. We investigated the impact of different initial population size (100 or 1000 mink) and simulated remediation efforts that varied in effectiveness (2.22 – 100%), frequency, and timing after the contamination event (5 – 20 years). Simulations of a baseline, uncontaminated mink population had no extinction risk while extinction risk for the PCB contaminated population was 1.0 in the absence of any clean-up. Initial population size had a strong impact on mink population dynamics with a greater extinction risk associated with an initially smaller population. In addition, mink populations were extremely responsive to clean-up scenarios that were initiated soon after the contamination event. In fact, the rate of PCB clean-up did not have as strong a positive effect on mink as did the initiation of clean-up (start time). There was also a beneficial effect of cleaning up “hot spots” compared to uniform PCB removal. Population-level approaches can be used to not only understand adverse effects of contamination but to also explore the potential benefits of various remediation strategies.

**WP094 Stakeholder Engagement in Dredged Material Management Decisions in Long Island Sound** Z. Collier, M. Bates, E. Chu, US Army Corps of Engineers, Engineer Research & Development Center; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center; T. Fredette, US Army Corps of Engineers, Engineer Research & Development Center; M. Habel, S. Wolf, M. Keegan, US Army Corps of Engineers, New England District. Typically, decisions regarding the method and location for the disposal of dredged materials have considered site-specific and engineering constraints such as capacity, funding, and transportation, and environmental considerations, such as risk, while only integrating views held by local stakeholders in an ad-hoc manner. Lack of transparency in stakeholder process could result in conflicts and biased decisions. For example, the decision of where to place dredged materials in Long Island Sound has historically been highly politically charged between New York and Connecticut. In fact, the US Army Corps of Engineers is in the process of redoing the Dredged Material Management Plan, costing millions of dollars, due to dissatisfaction among the various stakeholders with the initial proposal. Clearly, a method for integrating stakeholders into the decision making process is required if a mutually acceptable decision is to be reached. This presentation will outline a methodology in which values were elicited from stakeholders representing competing interests and applied to the placement of dredged materials in Long Island Sound. Stakeholders included representatives from government agencies, economic associations and environmental groups from New York and Connecticut. Through a series of interactive working groups and interviews, a comprehensive list of criteria, sub-criteria, and metrics was built, and values were gathered regarding tradeoffs between risks and benefits related to environmental, ecological, economic, social welfare, and human health and safety impacts. A model was built in which disposal sites

for dredged materials were prioritized based on both site-specific constraints as well as stakeholder values.

**WP095 *Prymnesium parvum*, An Emerging Threat to Inland Waters**

**B.W. Brooks**, Baylor Univ, Environmental Science and Biomedical Studies, Baylor Univ, Dept of Environmental Science; **J.P. Grover**, Univ of Texas at Arlington, Professor; **D. Roelke**, Texas A&M Univ. The Cuyahoga River. Sandoz and the Rhine River. *Exxon Valdez*. For most, reading these names triggers thoughts of human activities producing immediate impacts on aquatic life. In many countries, environmental regulatory frameworks have developed to support risk assessment and management approaches addressing prospective and retrospective risks of chemical contaminants to ecological receptors and human health. When unacceptable risks are identified, a variety of management efforts may be employed. Such approaches are not as widely defined or employed, however, for harmful algal blooms (HABs), whose increase in frequency and magnitude has occurred globally, resulting in deleterious effects ranging from fish kills to human health risks. Though the majority of HAB research has occurred in marine systems, cyanobacterial HABs are increasingly reported in inland waters. Unfortunately, environmental conditions leading to HABs are complex and often species specific, making it difficult if not impossible to develop a universal approach to management. Since 2000, HABs of *Prymnesium parvum* (golden algae), an invasive haptophyte historically examined in estuarine and coastal habitats, have increasingly produced devastating fish kills in inland waters of the United States. Managers no longer stock sport fish in some impoundments due to repeated, fish-killing *P. parvum* blooms. Seasonal *P. parvum* blooms in water supply reservoirs have resulted in cessation of municipal drinking water withdrawals. The Sandoz and the *Exxon Valdez* spills represent more widely publicized environmental disasters than the increased prevalence of *P. parvum* or other HABs in inland waters. However, recurrent golden algae blooms may be a much larger problem, particularly in light of climate change that might exacerbate the severity of blooms. In contrast to environmental management strategies for chemicals, a more complex web of chemical, physical and biological factors influences the site-specific risks of *P. parvum* to aquatic life. Consequently, challenges to existing ecological risk assessment and management paradigms for aquatic life use protection are more difficult. Here we examine mechanisms associated with the increased prevalence of *P. parvum* blooms and their impacts to aquatic life. Specifically, we identify challenges *P. parvum* presents to integrated watershed assessment and management strategies, and provide future perspectives and recommendations for research needs.

**WP097 Toxicity of an Uncharacterized Neurotoxin Linked to Avian Vacuolar Myelinopathy (AVM) to Algae, Crustaceans and Fish**

**J. Her-rin**, Univ of Georgia, Warnell School of Forestry and Natural Resources; **R. Bringolf**, Univ of Georgia, Interdisciplinary Toxicology; **R. Haynie**, S. Wilde, Univ of Georgia, Warnell School of Forestry and Natural Resources. Avian vacuolar myelinopathy (AVM) is an often lethal neurological disease primarily affecting American coots (*Fulica americana*) and bald eagles (*Haliaeetus leucocephalus*) in reservoirs and impoundments throughout the southeastern United States. Affected birds lack coordination and display characteristic lesions in the white matter of the central nervous system. The disease is a seasonal phenomenon, with observations of clinical signs and mortalities occurring from late autumn through the winter. All affected water bodies support robust populations of exotic submerged aquatic vegetation (SAV)-predominantly *Hydrilla verticillata*, *Egeria densa* and *Myriophyllum spicatum*-and a previously undescribed epiphytic cyanobacterium. The disease agent is theorized to be a neurotoxin produced by the cyanobacterium and consumed with SAV by herbivorous waterbirds. The toxin is transferred up the food chain when neurologically impaired waterbirds are consumed by bald eagles. Previous investigations indicate that the toxin is water soluble and that methanol is a suitable solvent for extraction of the toxin from SAV. To date, toxicity of extracts has been confirmed in avian gavage trials after examining brain tissue for characteristic lesions. Suspect lesions have also been observed in triploid grass carp fed SAV from an affected site. We hypothesize that the extract, derived from SAV collected during a winter 2010 epizootic, will elicit a response in other aquatic biota. We used standard toxicity tests, EPA Methods 1001.0, 1002.0 and a modified 1003.0 to determine the toxicity of the extract to fathead minnow (*Pimephales promelas*), *Ceriodaphnia dubia* and *Nostoc* sp., respectively. These tests are 7-day static renewal exposures with endpoints measuring survival, reproduction and changes in algal biomass. The results will be used to forecast

vulnerable populations, other than waterbirds and bald eagles, at affected sites. We discuss the utility of the test species for developing a bioassay for the detection of the AVM toxin without using an avian model.

**WP098 Chronic Toxicity of Microcystis aeruginosa in Fish: Unexpected Effects and Potential Implications**

**J. Park**, Univ of Tennessee, Center for Environmental Biotechnology; **E.D. Rogers**, Univ of Tennessee, Center for Environmental Biotechnology, Dept of Forestry, Wildlife and Fisheries, The Univ of Tennessee, Dept of Forestry, Wildlife and Fisheries; **M.J. Twiner**, The Univ of Michigan Dearborn, Dept of Natural Sciences; **T. Henry**, Univ of Plymouth, School of Biomedical and Biological Sciences, Univ of Plymouth, School of Biology, Univ of Plymouth, School of Biological Sciences; **S.W. Wilhelm**, Univ of Tennessee, Center for Environmental Biotechnology, Dept of Microbiology. Blooms of the harmful cyanobacteria *Microcystis* sp. occur in many freshwaters and persistence of the environmental conditions that favor blooms can cause chronic exposure of organisms to microcystin (MC) toxins. We investigated effects of environmentally relevant chronic concentrations of MC-LR and lyophilized *Microcystis aeruginosa* in aqueous and dietary exposures in fish and evaluated changes in gene expression, reproduction, and tissue histopathology. After 96-h exposure of larval zebrafish to either MC-LR (0, 100, 1000 ppb) or lyophilized *M. aeruginosa* (4.5 ppb MC-LR) analysis of changes in global gene expression (Affymetrix GeneChip® Zebrafish genome arrays) revealed changes in expression in genes known to be associated with MC exposure and in some genes of particular interest that we not associated with exposure to MC-LR (i.e., unique to *M. aeruginosa* treatment). Among these genes were the vitellogenin genes (*vtg*), which were highly up-regulated and indicative of the presence of an estrogenic substance being produced by the cyanobacteria and the potential for endocrine disruption in fish. Subsequently, we investigated chronic exposure to *M. aeruginosa* in adult zebrafish and evaluated reproduction. No effects were observed on number of embryos, hatching rate, or survival of larvae after 3-week exposure, and male fish did not have induction of *vtg*. Further experimentation with chronic aqueous and dietary exposure to *M. aeruginosa* or MC-LR in channel catfish *Ictalurus punctatus* indicated induction of *vtg* in some fish exposed through diet to *M. aeruginosa*. These results indicate that *M. aeruginosa* can produce a substance(s) with estrogenic properties, but the conditions which this substance(s) is produced are complex and unpredictable. Further experimentation to culture the cyanobacteria to elucidate conditions favoring production of estrogenic substance(s) and fractionation of the algae into components to identify the substance(s) are underway.

**WP099 Response of Freshwater Harmful Algae to Aquatic Nutrients in Qinshan Lake of Taihu Watershed, China**

**J. Zhang**, W. Ni, Zhejiang Univ; **Y. Luo**, The Third Institute of Oceanography, State Oceanic Administration; **J. Stevenson**, Center for Water Sciences, Michigan State Univ. The harmful algal blooms in the Lake Taihu that occurred in 2007 were reported worldwide. This study was designed to understand how nutrients, such as TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and TP, influence the growth and species of freshwater algae. Our research site was in Qinshan Lake, which is located in the headwaters of the Taihu watershed. Water and algal samples were collected monthly from ten sites of Qinshan Lake during 2008-2009. The results showed that average TN concentration was 4.47 mg/L, with 92.2% of sampling sites exceeding the TN standard. Average TP was 0.051 mg/L, with 37.9% of sampling sites exceeding the TP standard. The average Trophic Level Index (TLI) of Qinshan Lake was 53, which indicated the beginning stages of the eutrophic conditions. Average chl-a concentration was 12.83 mg/m<sup>3</sup>. Abundance of green algae and diatoms far outweighed other freshwater algae, and they dominated during most of year with the highest relative abundances of which was 96% and 99%, respectively. During the summer, blue-green algae became the most dominant with a maximum relative abundance of 69%. The blue-green algae were composed mainly of potentially toxic taxa, such as *Microcystis* sp., *Nostoc* sp. and *Oscillatoria* sp. During the winter season, dinoflagellates were the dominant taxa, with the highest relative abundance of 89%. Our study suggested that nutrients were the main factor regulating population growth of blue-green algae, and control of ammonia and covarying nutrient loads are the key solution for the prevention of algal blooms in Qinshan Lake of Taihu watershed.

**WP100 A Watershed Focus on Harmful Algal Blooms: Undergraduate, Graduate Student and Stakeholder Engagement**

**M.T. Homsher**, The Univ of Findlay, Environmental Safety & Occupational Health Dept, Univ of Findlay, Environmental Safety & Occupational Health Dept; **B.**



Dolan, Univ of Findlay; C. Norris, The Univ of Findlay, Chemistry Dept; A. Shepherd, The University of Findlay, Pharmacology; P. Martin, Blanchard River Watershed Partnership, Watershed Coordinator. Collaborators from The Univ of Findlay College of Science Biology, Chemistry, Environmental Health and Safety, and the Blanchard River Watershed Partnership are conducting macro-invertebrate surveys, phosphorus, nitrate/nitrite, *E. coli* screens, Suspended Solids, and Total Dissolved Solids studies to identify potential contribution sources to harmful algal blooms within the Lye Creek Area of the Blanchard River Watershed. We have nurtured this collaboration since 2004 when planning for the State of Ohio EPA Total Maximum Daily Load investigations. Regional watershed partners share expertise and creative solutions to resolve our watershed deficiencies. The Univ of Findlay conducts two annual Blanchard River cleanups supported by our undergraduate, and graduate students, faculty volunteers and local stakeholders. We have engaged with three volunteer service grants in the last six years to engage Findlay Central Middle School six grade students, Findlay, High School students, and, to conduct public watershed forum's within our six country watershed group. The 2010 Spring and Summer Harmful Algal Blooms of Lake St. Mary's and other Ohio locales, caused an estimated recreation venue loss of 100 million dollars. Our watershed action plan and education committee strives to coordinate a watershed proactive approach to these issues to improve our watershed. Every living organism in a watershed requires water.

**WP101 Elucidating Trichloroethylene Toxicity Mechanisms** V. De La Rosa, UC Berkeley, Nutritional Sciences and Molecular Toxicology; C. Vulpe, Univ of California – Berkeley, Nutritional Science and Toxicology. Trichloroethylene (TCE) is an industrial solvent that has been used since the early 20<sup>th</sup> century as a replacement for chloroform and ether in many industrial processes. Improper disposal of TCE has led to major drinking water contamination and increased public health concern. The toxicity of TCE has long been studied, but findings are controversial and inconsistent. Animal studies have shown that TCE is metabolized to the reactive metabolites DCVG and DCVC, which are responsible for adverse effects such as liver and kidney tumors, mitochondrial toxicity and apoptosis. Still, the molecular events underlying these endpoints remain unknown or debated. Using a functional genomics approach in the model organism *S. cerevisiae*, we aim to obtain a better understanding of the pathways involved in TCE toxicity by analyzing the metabolites DCVG and DCVC. Many of the metabolic pathways, stress response pathways and gene functions are conserved from yeast to higher eukaryotes, permitting the results from this analysis to inform studies in higher organisms. The IC<sub>20</sub> for dichlorovinyl glutathione (DCVG) and dichlorovinyl cysteine (DCVC) in yeast were determined to be 6.7 $\mu$ M and 18.3 $\mu$ M, respectively. These doses were used for acute exposures, followed by parallel deletion analysis. Preliminary data for DCVG shows an enrichment of genes involved in DNA repair, signaling cascades and autophagy. Confirmations of DNA repair genes *rad6*, *rad18* and *rad9* showed increased sensitivity to DCVG implicating DNA damage as a possible mechanism of toxicity. These genes function as critical components of translesion synthesis in yeast and humans, implying that DCVG may cause DNA lesions that cannot be repaired or hinder replication, leading to cell death. Other results to be presented include a comparison of DCVG and DCVC gene profiles to determine if the two metabolites act via similar mechanisms and DNA repair studies conducted in DT40 cells. Altogether these results provide a better understanding of trichloroethylene toxicity mechanisms in yeast and insight on possible TCE toxicity mechanisms in humans.

**WP102 Using Yeast Functional Toxicogenomics to Decipher the Toxicity of Organochlorinated Pesticides** B. Gaytan, Univ of California – Berkeley, Nutritional Science and Toxicology, UC Berkeley, Nutritional Science and Toxicology; A. Loguinov, Univ of California – Berkeley, Nutritional Science and Toxicology; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology; C. Vulpe, Univ of California – Berkeley, Nutritional Science and Toxicology. Exposure to organochlorinated pesticides (OCPs) has been linked to neurotoxicity, endocrine disruption, and cancer, but the cellular mechanisms of toxicity behind these effects remain largely unknown. It was hypothesized that a chemical genomics approach using a *Saccharomyces cerevisiae* gene deletion library could help elucidate the cellular mechanisms by which various OCPs induce toxicity. Pools of deletion strains were exposed in triplicate for five and fifteen generations to the IC<sub>20</sub>, 50% IC<sub>20</sub>, and 25% IC<sub>20</sub> OCP concentrations. The oligo sequences unique to each deletion

strain were PCR-amplified and hybridized to TAG4 arrays to identify sensitive, unaffected, and resistant strains. The overrepresented biological terms within the data assisted in the selection of individual deletion strains for growth curve experiments. It is demonstrated here that genes involved in transcriptional elongation, nitrogen utilization, and amino acid sensing are necessary for resistance to the toxaphene OCP, a profile very similar to that for the GMP synthesis inhibitors 6-azauracil and mycophenolic acid. Preliminary analyses for the dieldrin OCP indicate that autophagy and components of the pyruvate dehydrogenase complex are critical for cell survival under dieldrin exposure. Future investigations will refine the mechanism(s) in yeast and perhaps examine how the knockout or knockdown of orthologs in higher organisms, such as *C. elegans* or human cell lines, affects OCP toxicity.

**WP103 Biology-Environmental Health Science Nexus: Inquiry, Content and Communication** R. Hesselbach, Univ of Wisconsin-Milwaukee; C. Berg, Univ of Wisconsin-Milwaukee, Curriculum and Instruction; M. Carvan, Univ of Wisconsin-Milwaukee, Great Lakes Water Institute; L. Petering, H. Tomasiewicz, Univ of Wisconsin-Milwaukee; D. Weber, Univ of Wisconsin-Milwaukee, Childrens Environmental Health Sciences Center, Univ of Wisconsin-Milwaukee, Great Lakes Water Institute, Univ of Wisconsin-Milwaukee; D. Petering, Univ of Wisconsin-Milwaukee, Chemistry/Biochemistry. We have developed an inquiry-based program for high school environmental science and biology students. The objective of the program is to provide students with multiple, integrated opportunities to conduct authentic experiments that link biological concepts with environmental health issues. The aim is for students to learn through the process of scientific discovery, thereby gaining not only specific knowledge and interest in biomedical science, but also to begin to acquire the critical skills necessary to effectively assess and understand the empirical world. Specifically, students studied the effects of various environmental agents on zebrafish embryo development, earthworm behavior, and fathead minnow reproduction, which served as models for human health effects. Integrated in the modules were opportunities for students to write peer review research papers, communicate their results within and between science classes, and participate in an annual student research conference modeled after the format used in professional society meetings presenting their findings via oral presentations and posters. At this conference, professional scientists, community members, and Univ graduate students were in attendance and also shared their knowledge in the poster session. By this process, students had a unique opportunity to personally interact with researchers and community leaders in a professional manner. Teachers were provided with in-depth professional development experiences through a comprehensive summer training workshop, and year-long scientific and educational support by UWM faculty and scientists at the UWM Great Lakes WATER Institute. The summer workshop has 3 goals: 1) to provide a mini-course in environmental health concepts, 2) to engage the teachers in a hands-on experience with the modules they will be using with their students and 3) to develop the pedagogical skills necessary for successful completion of the standards-based experimental modules. Inherent to the program is the systematic evaluation process to assess the various facets of the project. (Support: NIEHS and NCRR 1R25RR026299-01)

**WP104 Laboratory-based Research Immersion Experiences: Creating Environmental Scientists in a Single Semester** C. Broussard, The Univ of La Verne, Univ of La Verne, Biology, Univ of La Verne, Professor of Biology. Research experiences for undergraduates are among the most effective tools to recruit, retain, and promote the success of undergraduates in science, technology, engineering, and mathematics (STEM), particularly for underprepared and under represented groups in STEM. In addition to retaining students in college, research immersion experiences can galvanize undergraduate interest in pursuing STEM careers, including research. A recent challenge has been to provide research opportunities in light of diminished budgets and shifts in fiscal priorities away from laboratory experiences for undergraduates. Given these challenges, how can the environmental science community expose undergraduates to research in environmental science and encourage them to pursue graduate studies in the field? In the current project, laboratory-based research immersion experiences were designed to introduce undergraduates to environmental science research and to actively involve undergraduates in emerging contaminant research using developmental biology as a lens to focus their efforts. We will concentrate on the overall design and how other institutions can adopt the model developed

at La Verne. This research was supported by the Dept of Biology and NSF DUE 0632831. The opinions expressed in this work are solely the author's.

**WP105 A Game-based Pedagogy for Sustainability Ethics** T. Seager, Arizona State Univ, School of Sustainable Engineering & Built Environm; E. Selinger, Rochester Institute of Technology; S. Spierre, Arizona State Univ, School of Sustainability; J. Sadowski, Rochester Institute of Technology. Existing approaches to ethics education in environmental professions typically rely on a liberal arts approach of reading, discussing and writing about case studies abstracted from historical experience or hypothetical scenarios. However, this approach is of only limited interest to the typical engineering and science student that is accustomed to more experimental and experiential learning styles. This presentation describes a new approach to teaching ethics that immerses the students in four non-cooperative game theory problems abstracted from several salient problems in sustainability that have their origins in environmental economics: 1) environmental externalities, 2) Tragedy of the Commons, 3) weak versus strong sustainability, and 4) inter-generational equity. Each game problem consists of an educational module that can be delivered in series or independently, and is designed to engage each stage of the Kolb learning cycle. Modules have been tested at multiple Universities in a blended classroom and on-line format. The results suggest that students believe they gain deliberative, leadership, teamwork and moral reasoning skills by participating in the games. The presentation also discusses the implication of the game experiences for real-world environmental problems such as climate change negotiation.

**WP106 Online Teaching and Learning Environmental Toxicology** K. Dixon, Texas Tech Univ, TIEHH; H. Zhao, Texas Tech Univ, Teaching, Learning, and Technology Center; H. Morrison, T. Tyagi, Texas Tech Univ. Teaching Environmental Toxicology courses over the internet offers students opportunities not available in the classroom course. Online learning can be synchronous, using tools such as chat, audio and video conferencing, and shared desktop. Online courses also can be offered asynchronously. That is, students can arrange their schedules to work on the course any time it is convenient for them. Asynchronous learning tools include e-mail, threaded discussion, and file attachments for assignments and exams. One of the greatest advantages of online teaching is that the online course is more accessible, especially for non-traditional students. Students do not have to attend regularly scheduled classes on campus. Because the number of environmental toxicology programs still is relatively small, access to environmental toxicology courses is limited. Teaching online means that non-resident students, including out-of-state and international students, will be able to take these courses. A second advantage of the online course is its flexibility, particularly in asynchronous learning. This flexibility also makes the course more accessible, allowing more students to take the course. A course can be designed to grade assignments and exams automatically. This requires a significant amount of time in course design and, because exam questions generally are limited to short answers, may not be appropriate for graduate level courses. We found that distributing assignments and exams as attachments works best. Likewise, the student's answers to exam questions and assignments can be "turned in" as attachments, either in an e-mail or using online teaching software. With the exception of wet lab courses, an entire curriculum in environmental toxicology could be offered online.

**WP107 Impacts of Mining-derived Metals on Riffle-dwelling Crayfish in the Tri-State Mining District of Southwestern Missouri and South-eastern Kansas, USA** A.L. Allert, USGS – Biological Resources Division, Columbia Environmental Research Center, USGS – Columbia Environmental Research Center, Biological Resources Division; R.J. DiStefano, Missouri Dept of Conservation; C.J. Schmitt, US Geological Survey, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; J. Fairchild, USGS – Biological Resources Division, Columbia Environmental Research Center; W. Brumbaugh, US Geological Survey, Columbia Environmental Research Center. The Tri-State Mining District, USA was one of the largest producers of lead-zinc ore in the world. Previous field and laboratory studies of this area found evidence of aquatic biota exposure to metals and documented toxicity of sediment to benthic invertebrates. Studies in two other Missouri lead-zinc mining areas documented impacts of mining-derived metals on crayfish populations and on caged crayfish in situ exposures. We sampled riffle-dwelling crayfish communities at 16 sites in three tributaries of the Spring River in Missouri and Kansas. Crayfish species richness, crayfish density, physical habitat,

and water quality were examined at each site to assess the ecological effects of mining-derived metals. Metals (Ni, Cu, Zn, Cd, Pb) were analyzed in surface water, sediment, detritus, and crayfish. Four species of crayfish were collected during the study, with *Orconectes neglectus* being the predominant species at all sites. *Orconectes medius* was found only at headwater sites in two of the tributaries. Mean crayfish densities were significantly lower at sites directly downstream of areas with deposits of mine wastes (i.e., chat and tailings piles). Also, mining-derived metals in water, sediment, detritus, and crayfish were significantly higher at these sites. Additional analyses will be conducted to investigate whether crayfish densities are primarily associated with concentrations of mining-derived metals, physical habitat or water quality. Potential adverse effects to wildlife and ecosystem function may result from the consumption of metals-contaminated crayfish and from the absence of the predominant shredder in Ozark streams.

**WP108 Evidence of Exposure to the Montara Oil Spill in Timor Sea Wildlife** M. Gagnon, Curtin Univ, Dept of Environment and Agriculture, Curtin Univ (Bentley Campus), Dept of Environment and Agriculture, Curtin Univ, Environment and Agriculture; C. Rawson, Curtin Univ, Dept of Environment and Agriculture. From August to October 2009 the Montara well in the Timor Sea released crude oil and gas condensate to the surrounding environment causing concern over the impacts on wildlife and commercial fisheries. During and immediately after the well hydrocarbon release, a number of deceased animals suspected of being impacted by the release were collected from the region along with a small number of live animals which later died in captivity from unknown causes. Necropsies were collected from 16 birds (14 common noddies, 1 lesser frigatebird, 1 wedge-tailed shearwater), one spotted sea snake, one horned sea snake and one green turtle. A total of 49 tissue samples and 39 swab samples were analysed for the presence of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons. One common noddie collected deceased from the vicinity of the Montara incident had crude oil on its feathers, in its gizzard contents, in its lungs and in its breast tissue indicating significant pre- and/or post-mortem exposure to crude oil. One common noddie collected at Ashmore Reef had crude oil in its lungs but in no other samples (e.g., feathers, trachea etc). It is likely that this indicates non-recent exposure. While the spotted sea snake had hydrocarbons in its lung only, the horned sea snake had crude oil in its stomach only and none in any other tissues (skin, trachea, lung). The presence of oil in the stomach of the horned sea snake suggests exposure through the ingestion of prey and not through surfacing in an oil contaminated environment. There was no evidence that the sea turtle had been exposed to crude oil either pre- or post mortem. Results indicate that across the wide geographical area where animals were collected, oil exposure might have contributed to lethality in only a few cases.

**WP109 Nearshore Avian Densities in the Northern Gulf of Mexico: Changes from May 2010 to May 2011** J.R. Wakefield, L. Noel, K. Gable, S. Fidler, P. Reilly, Cardno Entrix. A common refrain at SETAC's Gulf Oil Spill Focused Topic Meeting was the absence of studies designed to evaluate survival and reproduction among higher trophic organisms such as birds or marine mammals that may have been impacted by the spill. Studies conducted by Cardno ENTRIX scientists in 2010 and 2011 directly address this concern. From May 9 to May 15, 2010, BP representatives conducted comprehensive helicopter-based bird counts and photo-documentation along all coastal beaches, barrier islands, major bays, and marshes located between Venice, Louisiana and Pensacola, Florida. Collected days or weeks before oil came ashore, these observations include observations of nearly all major colonial water bird breeding colonies in the northern Gulf of Mexico and provide a comprehensive snapshot of pre-impact avian density in the nearshore environment. The overflights were repeated one year later (May 10 to May 15, 2011) and the data from the two sets of overflights provide the first comprehensive snapshot of pre- and post-accident avian populations; variation between these snapshots reflects both natural environmental variation as well as the integrated effect of oil and oil spill related changes in the avian prey base and habitats. Data from overflights will be depicted in a series of maps illustrating avian density and species composition in approximately 275 grid cells distributed throughout the northern Gulf of Mexico. These data and any variation between 2010 and 2011 will be placed in the context of time series data describing the magnitude of, and variation in, Louisiana's breeding bird densities during the past 15 years.

**WP110 Provisioning of Chicks by Leach's Storm-Petrels: Preliminary Insights into Energy Content, Lipid Content, and Contaminants of Stomach Oils** S. Camilleri, Univ of North Carolina Wilmington; H. Koopman, Univ of North Carolina Wilmington, Dept of Biology and Marine Biology; A. Westgate, Univ of North Carolina Wilmington, Dept of Biology and Marine Biology, Grand Manan Whale and Seabird Research Station; D. Gannon, Bowdoin Collete, Bowdoin Scientific Station c/o Dept of Biology; R. Mauck, Kenyon College, Dept of Biology; J.R. Kucklick, National Institute of Standards and Technology (NIST), Analytical Chemistry Division, National Institute of Standards & Technology, Hollings Marine Laboratory. Many adult Procellariiformes provision chicks with oils concentrated from consumed prey in contrast to most seabirds that supply chicks with whole prey. This feeding strategy is considered adaptive because chicks are provided with relatively higher energy lipids to better sustain them between parental foraging trips. Consequently, chicks may be ingesting higher concentrations of persistent organic pollutants (POPs) than if they ingested whole prey. Our goal was to evaluate the "oil" strategy by determining the energy content, lipid composition and organic contaminant concentrations in stomach oils of Leach's storm-petrels (LHSPs; *Oceanodroma leucorhoa*) from the Bay of Fundy, Canada and compare these with similar measurements from whole prey items. Samples collected in 2010 (n=83) indicate that the stomach oils are very energy dense (mean 35.18 kJ/g, SD=6.86, n=27). Wet weight values for potential prey items of other seabirds (herring 7,487 J/g; copepods 6,316 J/g) suggest that adult LHSPs can produce a significantly concentrated form of energy in stomach oils over the raw ingested prey items. Stomach oil (n=34) were comprised mostly of triacylglycerols (mean 73% SD=32.3) and wax esters (mean 25%, SD=30.84), however there was a wide range (0 to 90.2) of wax ester content, providing the first information that there is variation in diet among individuals. Stomach oil samples were analyzed for polychlorinated biphenyl congeners (PCB), DDT and related compounds, polybrominated diphenyl ether congeners (PBDE), chlorobenzenes, and mirex using gas chromatography mass spectrometry. Preliminary contaminant analyses of stomach oils (n=27) revealed ΣPCB levels ranging from 143-2433 ng/g lipid weight (median 590), ΣDDT levels ranging from 23-697 ng/g (median 161). Mirex ranged from 0.7-8.4 (median 2), HCB ranged from 1.7-41.9 ng/g (median 16), and oxychlordane ranged from 2-21 ng/g (median 7). These values are higher than published values (wet weight) for zooplankton collected from the same area (ΣPCB 114-241 ppb, ΣDDT 14-23 ppb), suggesting that stomach oils concentrate contaminants; consequently chicks may be receiving oils with enhanced organochlorine concentrations compared to whole zooplankton and other prey items.

**WP111 Footprint of Mercury in Terrestrial Songbirds Along the South Fork Shenandoah River** A. Jackson, D. Evers, Biodiversity Research Institute; A. Condon, US Fish and Wildlife Service, US Fish and Wildlife Service, US Fish and Wildlife Service; J. Schmerfeld, US Fish and Wildlife Service; D.A. Cristol, College of William & Mary, Professor of Biology, College of William & Mary, Dept of Biology. Mercury (Hg) is a persistent environmental contaminant found in many freshwater and marine ecosystems. In riverine systems, historical Hg contamination impacts the surrounding terrestrial ecosystem, but there is little known about how far downstream this contamination occurs. As part of a Natural Resource Damage Assessment, in 2009, we sampled terrestrial forest songbirds at five sites up to 137 kilometers downstream of the historical source of Hg along the South and South Fork Shenandoah Rivers (Virginia, USA). We found that blood total Hg concentrations remained elevated over the entire sampling area and there was little evidence of decline with distance. We also found that blood mercury concentrations varied based on foraging guild (invertebrate or omnivore) and migration strategy (resident or migrant). While it is well known that Hg is a pervasive and long-lasting aquatic contaminant, it has only been recently recognized that it also biomagnifies effectively in floodplain forest food webs. This study extends the area of concern for terrestrial habitats near contaminated rivers for more than 100 kilometers downstream.

**WP112 Acute Oral Lead Pellet Exposure in Adult Domestic Pigeons: A 28 Days Study** J. Holladay, M. Nisanian, S. Holladay, R. Kerr, Univ of Georgia, Anatomy and Radiology; L. Tannenbaum, US Army Public Health Command, Environmental Health Risk Assessment Program, US Army Institute of Public Health; S. Williams, Univ of Georgia, Poultry Diagnostic and Research Center; R. Gogal, Univ of Georgia, College of Vet Med, Anatomy and Radiology. Avian wildlife and domestic species commonly

ingest lead (Pb) spent shot or bullet fragments as grit or mistakenly as food. In previous studies in our laboratory and others, the reported toxicities varied based on the avian species, diet, type and quantity of Pb ingested. In the current study, domestic pigeons were gavaged with 1, 2, or 3 Pb pellets and followed with weekly radiographs and blood physiologic endpoints for 28 days. Pellet retention decreased by roughly 50% per week as pellets were either absorbed or excreted except for week 4 where the numbers remained constant. Size of retained pellets visually decreased based over time. Birds dosed with a single pellet showed mean blood Pb levels over 80 times higher than those of the controls, supporting a measured level of Pb absorption. Unlike our previous quail studies, no overt clinical signs of toxicity were seen across dose. However, similar to the Pb pellet-exposed quail, plasma d-aminolevulinic acid dehydratase (d-ALAD) activity remained depressed, suggesting an altered hematological function in the Pb pellet-exposed pigeons. Still compared to the quail, domestic pigeons appeared to have a higher tolerance to the oral Pb pellets.

**WP113 Assessment of Population, Reproductive, and Health Impairments in Colonial Waterbirds Breeding in Michigan's Areas of Concern** K. Grasman, Calvin College, Dept of Biology, Calvin College, Dept of Biological; S. Fuhrman, A. Moore, W. VanDenHeuvel, Calvin College, Dept of Biology; L. Williams, US Fish and Wildlife Service, East Lansing Field Office. The purpose of this assessment was to investigate and monitor the effects of contaminants on the breeding population numbers, reproduction, and immunological health of fish-eating birds in the Saginaw Bay and Raisin River Areas of Concern (AOCs) as part of the Great Lakes Restoration Initiative-Fish and Wildlife Service AOC program. In the Saginaw Bay AOC, field studies were conducted during 2010 at two herring gulls colonies (the Confined Disposal Facility (CDF) in the southern bay and Little Charity Island in the outer bay), two Caspian tern colonies (the CDF and Charity Reef) and one black-crowned night heron colony (CDF). At the Raisin River AOC, studies were initiated on the herring gull colony at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie. Gull nests were marked during egg-laying, and embryonic viability was assessed during mid/late egg incubation using an embryonic viability detector sensitive to heartbeat and movement. Embryonic nonviability in herring gulls in the Saginaw Bay and Raisin River AOCs (4-5%) was somewhat higher than at reference sites (< 2%). Total nonviability was most influenced by early failed development on the CDF and infertility on L. Charity, whereas at Monroe nonviability was spread throughout development. Herring gull colonies in the Saginaw Bay AOC showed marginally good chick survival. Caspian tern colonies showed marginal to poor chick survival, which is of concern for this state-threatened species. The herring gull colony at the Raisin River AOC experienced a complete reproductive failure with almost no chicks surviving through mid June. The phytohemagglutinin (PHA) skin test for T-cell mediated immunity was suppressed significantly in young gulls, terns, and herons living on several islands in the Saginaw Bay AOC. In herring gulls mean responses in both Saginaw Bay colonies were suppressed 65-66% compared to the marine reference site and 50-52% relative to the Great Lakes reference site. Compared to reference data, mean responses were suppressed 31-50% in Caspian terns, and 33% in black-crowned night herons. Ongoing immunological, developmental, and reproductive impairments in fish-eating birds at these AOCs are consistent with previous studies on the effects of persistent pollutants such as PCBs in Great Lakes birds, although other stressors may also be contributing, especially to the reproductive effects.

**WP114 Dietary Exposure of Mink (*Mustela vison*) to Fish from the Upper Hudson River, New York, USA: Organ Mass and Pathology** S.J. Bursian, Michigan State Univ, Dept of Animal Science; J.W. Kern, R.E. Remington, Kern Statistical Services, Inc.; J.E. Link, Michigan State Univ, Dept of Animal Science; S.D. Fitzgerald, Michigan State Univ, Dept of Pathobiology and Diagnostic Investigation. The Hudson River is contaminated with polychlorinated biphenyls (PCBs) from Ft. Edward, NY to New York City. The predominant sources of PCBs to the upper Hudson River were 2 General Electric plants in Fort Edward and Hudson Falls, NY that manufactured electrical capacitors containing PCBs beginning in the 1940s. Reports spanning the past 20 years indicated that mink (*Mustela vison*) collected within 1 home range of the upper Hudson River contained concentrations of PCBs in their fat and livers comparable to concentrations causing reproductive impairment in controlled studies with ranch mink, and that tissue concentrations of PCBs have not decreased appreciably. Because the mink is a fish-eating mammal that satisfies criteria, including chemical



sensitivity, for a sentinel wildlife species, it is a commonly selected ecological receptor in studies at sites involving aquatic habitats with elevated concentrations of PCBs. A study was conducted to evaluate the health effects of feeding ranch mink diets containing PCB-contaminated fish from the Hudson River. The effects on adult and offspring organ mass and pathology are reported here. Diets contained 2.5% to 20% Hudson River fish, providing 0.72 to 6.2  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (5.1 to 38 pg toxic equivalents [TEQs]/g feed). Absolute thyroid gland and adrenal gland masses were increased in adults and 31-wk-old juveniles, respectively, and absolute liver and heart mass were decreased in 6-wk-old kits exposed to dietary PCBs. Dietary concentrations of 0.72  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (5.1 pg TEQs/g feed) and greater induced mandibular and maxillary squamous epithelial proliferation. The dietary concentration of  $\Sigma\text{PCBs}$  predicted to result in 20% incidence of the jaw lesion was 2.3  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  feed (15 pg TEQs/g feed) and the hepatic concentration was 2.8  $\mu\text{g}$   $\Sigma\text{PCBs/g}$  liver, wet wt (89 pg TEQs/g liver, wet wt). The conclusions and opinions presented here are those of the authors and do not represent the official position of any of the funding agencies, the Hudson River Trustees or the United States.

**WP115 Persistent Organic Pollutant (POP) Effects on Shovelnose Sturgeon (*Scaphirhynchus platyrhynchus*) Reproduction and Early Life Stages** L. Buckler, Univ of Missouri-Columbia, US Geological Survey Cooperative Research Unit, Univ of Missouri-Columbia, Fisheries and Wildlife; J. Candrl, US Geological Survey, Columbia Environmental Research Center; M. McKee, Missouri Dept of Conservation; D.M. Papoulias, D.E. Tillitt, US Geological Survey, Columbia Environmental Research Center; D. Galat, Univ of Missouri-Columbia. The genus *Scaphirhynchus* is endemic to the United States and includes the federally endangered pallid sturgeon (*Scaphirhynchus albus*). The more abundant shovelnose sturgeon (*Scaphirhynchus platyrhynchus*) is commonly used as a biological surrogate for pallid sturgeon. However, populations of both *Scaphirhynchus* species have been in decline over the past century. Shovelnose sturgeon have consumption advisories in five of the 13 states that list them as a sport species. These advisories are largely due to concentrations of persistent organic pollutants (POPs) found in muscle and roe tissues. Concentrations of these POPs are above levels shown to have adverse reproductive effects in other species. This study was designed to determine if concentrations of POPs in egg samples from wild fish, specifically organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs), affected early life stage development and survival in shovelnose sturgeon. Twenty-six female shovelnose sturgeon from two Middle Mississippi River sites were artificially spawned and contaminant concentrations in eggs were analyzed using gas chromatography. Percent fertilization, percent hatch, and percent survival to exogenous feeding were endpoints used to determine if POPs were affecting early life stages. Lipid normalized egg concentrations ranged from 540 – 2,560, 50 – 1,650, and 790 – 8,500 ng/g lipid for total OCPs, total PBDEs, and total PCBs respectively. Percent fertilization was not correlated with POP concentration. However, percent hatch ( $r = -0.42$ ,  $p = 0.03$ ) and cumulative percent survival at 2 ( $r = -0.42$ ,  $p = 0.03$ ), 3 ( $r = -0.41$ ,  $p = 0.04$ ), 4 ( $r = -0.48$ ,  $p = 0.01$ ), and 5 ( $r = -0.43$ ,  $p = 0.03$ ) days post fertilization (dpf) were inversely correlated with POP concentration. Furthermore, a total PCB LC<sub>50</sub> of 2,700 ng/g lipid was calculated for shovelnose sturgeon at 3 dpf. Results from this study indicate that recruitment of shovelnose sturgeon and potentially pallid sturgeon may be adversely effected at the upper extreme of POP concentrations observed in the field.

**WP116 Assessment of Potential Neosho Madtom Habitat in Tributaries of the Spring River of Kansas and Missouri** C.J. Schmitt, US Geological Survey, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center. The Neosho madtom (*Noturus placidus*: Pisces, Ictaluridae) is a Federally listed (Threatened) fish endemic to the Neosho-Spring River system of Kansas, Oklahoma, and Missouri. It presently occurs in the main stem of the Spring, Neosho, and Cottonwood rivers upstream of the Tri-State Mining District; at several Spring River main stem locations further downstream; and in a few Kansas tributaries of the Neosho River. Neosho madtoms also occurred historically in the Illinois River of Oklahoma, a Neosho River tributary. However, there is only one historical record of the species occurring in any of the Spring River tributaries flowing westward from Missouri and Kansas (Center, Turkey, and Shoal creeks), which have been contaminated to varying degrees by metals from historical lead-zinc mining. Because the Neosho madtom was not formally recognized as a species until 1969, 100 y after mining began, it is not known

whether some populations were extirpated. The objective of this study was to evaluate extant data to determine whether the westward-flowing tributaries could support Neosho madtoms absent mining-related contaminants. The approach was to apply a regression model developed from 1991 data for Neosho madtom density and habitat in the Neosho River system to more recently obtained tributary data, and to develop additional regression and principal components models based on all available habitat and fish data exclusive of contaminants. All the models indicated that the lower reaches of the westward-flowing tributaries could support Neosho madtoms. The results are consistent with extant information on the present and historical distribution of the species indicating that its range can expand and contract rapidly in response to flow and other habitat changes, and that it can tolerate a wide range of habitat conditions. Collectively, the results support the premise that Neosho madtoms inhabited the lower reaches of the tributaries at least occasionally prior to the advent of mining, and that re-establishment of populations in these streams is feasible absent contamination.

**WP117 Effects of Mining-Derived Metals on Crayfish in Several Mining Districts of the Central USA: A Review** A.L. Allert, USGS – Biological Resources Division, Columbia Environmental Research Center, USGS – Columbia Environmental Research Center, Biological Resources Division; J. Fairchild, USGS – Biological Resources Division, Columbia Environmental Research Center; R.J. DiStefano, Missouri Dept of Conservation; W. Brumbaugh, US Geological Survey, Columbia Environmental Research Center; C.J. Schmitt, US Geological Survey, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; J.M. Besser, US Geological Survey, Columbia Environmental Research Center. The abundant lead and zinc resources of Missouri, Kansas, and Oklahoma, USA have been mined since the 1700s, resulting in large quantities of metal-enriched mine tailings being deposited into some streams of the mining districts. Contamination of fish and other aquatic biota, alteration of fish and invertebrate communities, and public health advisories against human consumption of lead-contaminated fish have resulted. We summarize the findings of studies that documented the impacts of mining-derived metals on crayfish populations in the Viburnum Trend Mining District (VTMD), Old Lead Belt (OLB), and Tri-State Mining District (TSMD). Metal concentrations, especially lead, zinc, and cadmium were significantly higher in water, sediment, organic matter, and biota at mining sites compared to reference sites. Crayfish densities were significantly lower at sites directly downstream of mining areas compared to reference sites in the three mining districts. In situ toxicity to juvenile crayfish was significantly higher at mining sites compared to reference sites in the VTMD and OLB and provides direct evidence that mining-derived metals are toxic to crayfish. In the VTMD, organic matter processing by crayfish was significantly lower at mining sites than at reference sites. Crayfish are an important prey item for other aquatic and terrestrial invertebrates, amphibians, reptile, fish, birds, and mammals and are critical to organic matter processing and the cycling of nutrients and energy through stream food webs. Therefore, the effects of mining-derived metals on crayfish may impact the functional integrity of Ozark streams and surrounding ecosystems.

**WP118 Assessing Protection of EPA 304(a) Chronic Criteria Recommendations to Federally-listed Fishes** T.K. Linton, Great Lakes Environmental Center; W.K. Taulbee, Great Lakes Environmental Center, Research Scientist; C.M. Voros, Great Lakes Environmental Center; D. Lehmann, US Environmental Protection Agency, Health and Ecological Criteria Division, OST, OW; J. Beaman, USEPA, Toxicologist; F. Mayer, GLEC Subcontractor. Under section 304(a) of the Clean Water Act (CWA) the USEPA develops ambient water quality criteria that serve as scientific recommendations for states and tribes in adopting and revising water quality standards. Currently, EPA is required under section 7 of the Endangered Species Act (ESA), to consult with the US Fish and Wildlife Service (FWS) and the National Marine Fisheries Services (NMFS) (collectively, the Services) when approving new and revised state and tribal water quality standards (WQS) under section 303(c) of the CWA. EPA's aquatic life criteria are designed to provide for the protection and propagation of aquatic communities in general, inclusive of federally threatened and endangered aquatic species. The goal of this research is to develop and validate a new approach for these consultations that 1) ensures EPA's recommended criteria are protective where locally sensitive populations of Federally-listed aquatic species and their critical habitat occur, and 2) is simple, flexible, and scientifically defensible so that such an approach can be readily adopted by

states and tribes. The approach builds on an already strong foundation from other such studies published in the peer-reviewed literature where select surrogate species tested with a wide array of data-rich chemicals against Federally-listed fish species have proved both representative and useful. The overall approach incorporates multiple lines of evidence from comparative and probabilistic-based toxicology to derive and validate a simple "criterion adjustment factor" based on the use of the taxonomically-representative and chemically-sensitive surrogate taxa: *Oncorhynchus mykiss* (rainbow trout). The approach is being developed for several chemicals with different adverse outcome pathways (copper, ammonia, carbaryl, and 4-nonylphenol), using readily available predictive tools (e.g., interspecies correlation, acute:chronic estimator, species sensitivity distribution) and other scientifically-accepted conventions (e.g., acute-chronic ratios). Appropriate criterion adjustment factors derived with appropriate margins of safety based on a prescribed level of acceptable uncertainty will be presented.

**WP119 Toxicity of Portland Harbor Sediments to Pacific Lamprey Ammocoetes (*Lampetra tridentata*)** J.M. Morris, J. Peers, J. Lipton, Stratus Consulting; C. Schreck, R. Chitwood, J. Unrein, Oregon State Univ. Contaminants such as chlorinated hydrocarbons, petroleum-related compounds, metals, and other hazardous substances have been released from various sources into Portland Harbor and have accumulated in sediments. Sediments from specific areas in the Harbor are toxic to benthic invertebrates, and benthic organisms and fish in the Harbor have accumulated contaminants. Benthic habitat in the Harbor may be an important resting and foraging area for larval Pacific lamprey (*Lampetra tridentata*) as they transition to the lower Columbia River and undergo metamorphosis to their adult life stage. Larval lamprey (ammocoetes) in the Harbor likely are exposed to a variety of contaminants and ammocoetes collected from the Harbor have been found to have accumulated organochlorine compounds to a greater degree than ammocoetes collected from upstream areas. In support of the Portland Harbor Natural Resource Damage Assessment, this pilot project was aimed at developing experimental methodologies to assess effects of contaminated sediments on physiological and behavioral endpoints. Ammocoetes collected from the Siletz River, OR, were used to evaluate sensitivity to contaminated Portland Harbor sediments. Phase I of this project included a series of experiments focused on methods development and results were presented at the annual SETAC meeting in Portland in 2010. Phase II of this project included sediment bioassays and behavioral testing using sediments collected from 9 sites in Portland Harbor. The results of Phase II testing will be presented.

**WP120 Assessing Potential Injury for the Hanford Site NRDAR Using a Native Mussel** J. Bartoszek, US Fish and Wildlife Service, Division of Environmental Assessment and Restoration; R. MacRae, US Fish and Wildlife Service. The Hanford Reach of the Columbia River has received contaminants from the Dept of Energy Hanford site since the 1940s. Hexavalent chromium has been released to the river through both surface water and upwelling ground water pathways, and preliminary laboratory studies indicate that concentrations of hexavalent chromium similar to those measured or predicted in the river could negatively impact Chinook salmon and/or Steelhead trout. However, because of their mobile life history and impacts from habitat alteration and hydrologic changes, attributing injury to these fish due to release of contaminants from the Hanford site is complex. Thus, the Hanford Natural Resource Trustee Council is considering several alternative species to determine if injury to aquatic biota has occurred. The Hanford Reach of the Columbia River has historically supported populations of native mussels such as species of the genera *Anodonta* and *Margaritifera*. Although reduced in numbers, *Anodonta* (floaters) still occur in the Reach, whereas the once abundant *Margaritifera falcata* (western pearlshell) may be extirpated. While habitat modifications may have contributed to these declines, historical contaminant releases may have also played a role, particularly if the western pearlshell is more sensitive to contaminants than floaters. Because they are sessile and can be exposed to contaminants both from the water column and sediments, these native mussels will likely be the subject of injury studies.

**WP121 Can You Afford To Not Have Vegetation Data for Metals?** E. Curtis, AMEC Environment & Infrastructure, Project Manager – Ecological Risk Assessment; M. Bystedt, M. Bystedt, N. Ruberti, L. Stubblefield, AMEC Environment & Infrastructure. Bioaccumulation of metals into vegetation is not a typical pathway of quantification in ecological risk

assessment. Bioaccumulation factors (BAFs) and slopes provided in Empirical Models for the Uptake of Inorganic Chemicals from Soil by Plants (Bechtel, 1998) are often used for estimating metal concentrations in vegetation tissue in food-web modeling in the absence of site-specific plant tissue data. The vegetation BAFs in Bechtel (1998) are typical of literature based soil-to-biota bioaccumulation factors, but these values have a layer of conservatism, the upper prediction limit, built in for use at sites with no available plant data. The use of the upper predictive limit often leads to overestimation of metal concentrations in vegetative tissue. A site-specific plant bioaccumulation study on arsenic, cadmium, copper, lead, and zinc at a site in western Georgia was conducted using the approach presented in Bechtel's study. The site-specific dataset obtained from the plant bioaccumulation study was small with limited variability. A meaningful relationship (provided by R<sup>2</sup> values) between soil and tissue concentrations could not be determined. By combining the site-specific data with the vegetation BAFs developed from the larger dataset in Bechtel (1998), a more realistic quantification of risk for ecological receptors was determined than was provided by the use of the literature-based BAFs.

**WP122 Developing Effect-based Ecological Soil Screening Levels (EcoSSLs) for Use in Wildlife Terrestrial Baseline Ecological Risk Assessments** S. Pauwels, TechLaw, Inc.; R. Sugatt, USEPA Region 1; R. Dionne, E. Czerepak, TechLaw, Inc.; B. Hoskins, USEPA Region 1, New England Regional Laboratory. The Environmental Protection Agency (EPA) published Ecological Soil Screening Levels (EcoSSLs) protective of plants, soil invertebrates, birds, and/or mammals. These values represent contaminant levels in soil not expected to cause ecological harm. A limiting factor is that EcoSSLs are No Observed Adverse Effect Level (NOAEL) screening benchmarks. Such values can help identify contaminants of concern, but are less useful to quantify risk or support risk management decisions. This study developed Lowest Observed Adverse Effect Level (LOAEL) EcoSSLs for terrestrial birds and mammals. The first step was to obtain the EcoSSL documents for each target contaminant and identify the NOAEL and LOAEL Toxicity Reference Values (TRVs) for reproduction and growth which were unbounded (i.e., derived from a study providing either a no effect or an effect value, but not both), bounded (i.e., derived from a study providing both no effect and effect values), or a compilation of both types of values. The latter were referred to as "combined TRVs". These TRVs were then used to calculate a geometric mean TRV for the three sets of toxicity values (i.e., unbounded TRVs, bounded TRVs, and/or combined TRVs) and the two toxicity levels (i.e., NOAELs and/or LOAELs). The second step used the food chain model and exposure parameters from the EcoSSL reports to "back-calculate" chemical-specific LOAEL-based wildlife EcoSSLs. The six receptors used by EPA in the original calculations were retained for this project. These species were two herbivores (mourning dove and meadow vole), two soil invertivores (American woodcock and the short-tailed shrew) and two higher trophic-level carnivores (red-tailed hawk and the long-tailed weasel). The lowest of the chemical-specific values for birds and mammals was selected as the final "bird" and "mammal" LOAEL EcoSSL. The calculations showed that, with a few exceptions, the two soil invertivores had the lowest LOAEL EcoSSLs. The accuracy of the food chain model and exposure parameters was also double-checked by recalculating NOAEL EcoSSLs and comparing these values against the original NOAEL EcoSSLs. This internal check showed that our food chain model, exposure parameters, and calculations were correct within rounding error. The LOAEL EcoSSLs developed for this project have been used successfully in several baseline ecological risk assessments to better evaluate the range of risks to terrestrial wildlife receptors exposed to soil contaminants.

**WP123 Earthworm Behavior in Soils with Added Amendments, Various Agronomic Practices, and a Bioenergy Crop** J. Choate, J.W. Klasky, Arkansas State Univ, Environmental Sciences; J.L. Bouldin, Arkansas State Univ, Dept of Biological Sciences, Arkansas State Univ, Environmental Sciences Graduate Program, Environmental Sciences Graduate Program, Arkansas State Univ, Dept of Environmental Science. Earthworm avoidance tests have advantages for use in soils for the determination of soil health, structure, and toxicants present. Since earthworms show behavioral responses at a lower scale than acute toxicity tests, avoidance tests are sensitive indicators of low-level contaminants. This avoidance is an ecological endpoint that is relative to the potential of the substrate's toxicity to organisms. When toxicants are present, impacts on earthworms can be tested in a short time frame. The influence of pollution and toxicants on earthworm

populations not only changes behavior but can also have an impact on the soil ecosystem. The experiments were conducted separately using three different soil medias. First, control soil was obtained and locally available soil amendments were added before the earthworms were introduced. Second, soil from a sorghum field (bioenergy crop) with varying nitrogen sources was utilized as a field representation of earthworm behavior. Third, soil was obtained from a cotton field with three different agronomic management regimes (no-tillage, conventional tillage, and cover crop). Results from the bioenergy crop soils indicate earthworms congregating behavior which suggests a soil preference due to the availability of food. Sensitivity of this research was approximate to the sensitivity of established tests. For soil amendments, a considerably higher sensitivity was determined as compared to soils from bioenergy crops. In tests with a control soil spiked with reference toxicants, worms avoided this soil and a high mortality rate was observed.

**WP124 *Lobella sokamensis* (Collembola: Neanuridae) as a New Test Species to Assess Soil Toxicity** Y. An, Konkuk Univ, Dept of Environmental Science; S. Kim, Konkuk Univ; W. Lee, Konkuk Univ, Konkuk Univ, Dept of Environmental Science. Collembola are one of the most abundant groups in soil ecosystems. *Lobella sokamensis* (Deharveng et Weiner, 1984) is in the Neanuridae family, which is one of the most diverse families belonging to Collembola. It is an orange-colored springtail. *L. sokamensis* is endemic species of Korea, commonly found with earthworms. The present study suggested *Lobella sokamensis* as a new test species to assess soil toxicity. The acute toxicities of zinc, copper, cadmium, antimony, and mercury on the survival of adults were performed in artificial soil. *L. sokamensis* juveniles (10-12 day old) were also exposed to Cd, Cu, and Sb, and 5d-LC50 was calculated. The survival of adults and juveniles were inhibited on metal spiked soil, and we found that *L. sokamensis* juveniles were sensitive enough to assess toxicity of contaminated soil system, compared with *F. candida* assay. We observed that 5d-acute soil test using *L. sokamensis* juveniles can be rapid and cost-effective protocols to assess soil toxicity.

**WP125 Sensitivity Analysis on Terrestrial Risk Assessment Modeling to Aid the Development of Testing Strategy** M. Fan, The Procter & Gamble Company; F. Verdonck, ARCHE; S. Gimeno, The Procter & Gamble Company; T. Delfosse, The Procter & Gamble Company, Product Safety & Regulatory Affairs; P. Sun, The Procter & Gamble Company. While terrestrial assessments may be performed less frequently than aquatic assessments, industry seeks to continuously improve experimental tools, models, and risk assessment implementation. Substances which are most likely to be of concern in the terrestrial environment include those that are persistent and lipophilic. Within new chemical management schemes such as REACh, it is anticipated that more testing will be required for such substances when a screening (and conservative) assessment indicates risk that depends greatly on screening level physical-chemical properties for prioritization. To facilitate the development of testing strategy for parameters and then refining the assessment, a sensitivity analysis for terrestrial risk assessment was conducted using EUSES (European Union System for the Evaluation of Substances) model. The key parameters for the analysis included sorptivity, biodegradability, volatility, and ecotoxicity. The focused parameters and their probability distributions were selected based on a targeted literature review. The Monte Carlo based method outputs were summarized using Spearman rank order correlation coefficients and tornado diagrams to evaluate the sensitivity of parameters contributing to the Risk Characterization Ratio (RCR). The result indicated octanol-water partition coefficient (Kow) is the most influential chemical property. Further analysis for parameters having non-linear effects on RCR (i.e., Kow, biodegradability class, vapor pressure) revealed that the trend for such effect may vary between point sources industrial release and wide dispersive use scenarios (e.g., higher sensitivity of biodegradation to RCR for hydrophilic substance in wide dispersive use, but no remarkable difference for the point sources release). Finally, look-up tables were developed for each defined class of use scenario, biodegradability, Kow and vapor pressure, with a 90th percentile RCR derived for a quick easy-to-use screening assessment purpose. Testing strategy can then be developed by targeting the most meaningful parameters that will refine the assessment and ensure the safe use of those substances.

**WP126 Comprehensive Risk Assessment of a Proposed \$300M Cleanup Plan at Sydney, Nova Scotia** B. Magee, J. Keating-Connolly, ARCADIS; B. Chew, AMEC. Before implementing a comprehensive cleanup of 100

hectares of a former steel mill/coke oven complex, the Canadian Environmental Assessment Act required an Environmental Impact Statement, including a quantitative risk assessment of the proposed cleanup plan to ensure that the risks of the remedy did not pose unacceptable risks to human health and the environment. The risk assessment quantitated the risks of site constituents (benzene, toluene, ethylbenzene, xylenes, naphthalene, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and metals) and construction equipment related constituents (sulfur and nitrogen oxides, carbon monoxide, and fine and ultrafine particulate matter) associated with dozens of sources at dozens of specific locations. Sources included construction vehicle exhaust, dust from excavation, grading, transportation, loading and unloading, and stockpiling activities, and site-related volatile organic compounds from excavation, stabilization, and other activities. Because the proposed project was seven years in duration, many possible sequencing scenarios were evaluated to assess the impacts of different sources at different locations during different meteorological conditions. In addition, because the Final Design was not completed at the time of the assessment, different construction methods were assessed, such as ex situ versus in situ solidification/stabilization techniques and ambient stabilization versus stabilization in sprung structures to inform the Final Design. The risk assessment showed that the project could be completed safely, and it will be argued that performance of risk assessments during the design and evaluation of remedial planning can provide great value, especially at sites where the need for remedial actions is obvious. In addition, risk assessment is ongoing to ensure on a real time basis that remedial work is being done safely by the use of risk-based fence line stop work criteria and community health criteria.

**WP127 Ecological Risk Assessment & Site Management – Myths and Misunderstandings That Lead to Site Mismanagement** T. Rodolakis, AMEC. Potentially responsible parties and agency regulators look at the same data when evaluating a site. Yet much of the time, the two parties draw very different conclusions as to the level of ecological risk and how that risk should be managed. Spirited discussions often ensue because one party believes that the policies and practices used by the other party to interpret the data are misguided or misapplied. Since final management decisions can be costly in terms of dollars, damage to habitat, and unintended harm to other ecosystem assets, it is critical that those decisions are technically sound. This presentation examines the myths, misunderstandings, and technical merits behind 7 ecological risk assessment and risk management practices that have costly consequences when applied incorrectly.

**WP128 Residual Ecological Risk Assessment Case Study: Assessing Risk in Wetlands Habitat** K. Bradley, GEI Consultants, Inc., Ecologist; C. Claytor, GEI Consultants, Inc.; A. Leifer, GEI Consultants; R.W. Gensemer, GEI Consultants, Inc, Ecological Division; R. Helfeld, Massachusetts Dept of Conservation and Recreation. A case study will be presented reviewing the challenges faced in the investigation of ecological risks in support of the selection of remedial approaches at a historic airport site in Canton, Massachusetts. The site is planned for redevelopment as a recreational park. A region of the site deemed the "Hangar Area," was not included in the ecological risk assessment (ERA) due to high concentrations of PCBs resulting in presumed risks to human and ecological receptors requiring active remediation. A residual ERA, designed to meet the requirements of both the Massachusetts Contingency Plan and the Federal Toxic Substances Control Act, focused on the potential for ecological risks beyond the footprint of anticipated remediation throughout the wetland areas located on the site. The ERA process incorporated analytical sediment, surface water, soil and tissue data, a vegetative community assessment, and sediment toxicity data associated with these areas anticipated to remain post-remediation. Results of the residual risk characterization were evaluated using a weight of evidence procedure. The ERA identified a limited potential for residual risks to ecological receptors exposed to lead and zinc, and to a lesser extent, chromium, vanadium, and PCBs in site media. However, the overall confidence in the predicted risks at the site was only low to moderate based on the weight of evidence analysis. Ecologically based remediation goals (RGs) were proposed for PCBs by compiling all toxicity reference values used in the ERA and resolving each value to a protective soil or sediment concentration. Individual location RGs (protective of sessile organisms) were determined to be 0.7 mg/kg dw of PCBs in sediments and 25 mg/kg dw of PCBs in soils. Site-wide average RGs (protective of large-range wildlife receptors) were determined to be 90 mg/kg dw of PCBs in sediments and 50 mg/kg dw of PCBs in soils. Ultimate remedial decisions regarding portions of the site outside the



Hangar area will, however, require a balance between reduction of risk to ecological receptors as identified in the residual ERA with protection of valuable wetland habitats. The site is located within an area designated as an Area of Critical Environmental Concern (ACEC) by the Massachusetts Executive Office of Environmental Affairs.

**WP129 Will Ocean Acidification Affect Metal Uptake by the Marine Bivalve *Mytilus edulis*?** W.E. Robinson, Univ of Massachusetts-Boston, EEOS Dept; H.C. Poynton, US Environmental Protection Agency, Molecular Indicators Research, Univ of Massachusetts-Boston, Environmental, Earth and Ocean Sciences; R.E. Hannigan, Univ of Massachusetts-Boston, EEOS. Increasing atmospheric  $p\text{CO}_2$  has gradually lowered the pH of seawater from 8.2 in preindustrial times to 8.1 today. Some models predict that seawater pH could drop below 7.8 by 2100. These declines represent a 26 % increase in hydrogen ion concentration  $[\text{H}^+]$  since preindustrial times, and a 151 % increase in  $[\text{H}^+]$  by 2100. Studies of the biological consequences of this ocean acidification have primarily focused on reduced biocalcification (corals, pteropods, urchins and bivalves). However, an even more fundamental impact has received little attention – changes in metal speciation, uptake, and blood transport. The equilibrium speciation program MINTQA2 was used to calculate changes in seawater free ion activity for a number of divalent metals (e.g., Ca, Cd, Cu, Ni, Zn) under a range of pH values (8.2 to 7.8). While some metals exhibited increases in free ion activity (e.g.,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ), metals that are already predominantly present as free metal ion (e.g.,  $\text{Ca}^{2+}$ ) or that are complexed by chlorides (e.g.,  $\text{Cd}^{2+}$  or  $\text{Ni}^{2+}$ ) showed little change. Based on the Free Metal Ion, and the Biotic Ligand, models of metal uptake, we hypothesize that metals exhibiting an increase in free metal ion activity as a result of ocean acidification (e.g., Pb) will increase metal uptake whereas metals not exhibiting elevated free metal ion activity (e.g., Cd) will exhibit no change in uptake. To test this prediction, mussels are exposed to a mixture of metals (Cd + Pb) at three different pH levels (8.1 control, 7.8, 7.5). At Tzero, 15, 30 and 45 d, seven mussels will be dissected from each exposure tank, hemolymph removed, and the tissues analyzed for Cd and Pb concentrations. Hemolymph will be assayed for pH, protein, and free  $\text{Ca}^{2+}$  ion activity (ISE) in order to assess acid-base imbalance.

**WP130 Effects of Atmospheric  $\text{CO}_2$  on the Life Cycle and Fitness of the Mysid Shrimp *Americamysis bahia*** J.S. Grear, US Environmental Protection Agency, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, USEPA; D. Horowitz, US Environmental Protection Agency, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, USEPA, ORD, NHEERL, Atlantic Ecology Division; R. Gutjahr-Gobell, M. Bernardo, US Environmental Protection Agency, Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development. Most concern about effects of  $\text{CO}_2$ -induced ocean acidification focuses on mollusks, corals, and coccolithophores because skeletal and shell formation by these organisms is sensitive to the solubility of calcium minerals. However, many other marine organisms are likely affected by calcium availability and other acidification-related conditions such as altered cellular acid-base balance. We studied responses of the mysid *Americamysis bahia* under ambient and increased  $\text{CO}_2$  conditions in flow-through aquariums. This included bubbling ambient and  $\text{CO}_2$ -enriched air into aquarium seawater to simulate predicted increases in anthropogenic  $\text{CO}_2$ . Our experiments included a cohort study (initiated with 1-day old groups of individuals) as well as a 5-month study of demographic responses in intact, size-structured populations. The cohort study was intended to simulate a  $\text{CO}_2$  increase of 100-200 parts per million (partial pressure), which is within the range of that expected during the next century. Responses were monitored weekly by counting and sizing individuals. The strongest response was in the number of neonates present at the end of the third week, which was 3 times higher in the ambient control than in the  $\text{CO}_2$  treatment (5 aquaria per treatment; each aquarium initiated with ten 1-day old individuals). The demographic study was smaller (2 tanks per treatment) but ran for twenty weeks and produced ~7500 mysid length measurements. Effects were less clear, but combined with cohort results, suggest that ocean acidification has the potential to affect the demography of marine crustacean populations. Monitoring of carbonate system characteristics in our study was limited, so further studies are needed. These studies should include a gradient of realistic  $\text{CO}_2$  manipulations and should recognize the potential for differences between cohort-level and population-level responses.

**WP131 Impacts of Ocean Acidification on Copper Speciation and Toxicity** M. Grosell, Univ of Miami, RSMAS; K. Munley, A. Esbaugh, RSMAS, Univ of Miami; K.V. Brix, Univ of Miami, RSMAS, Marine Biology and Fisheries; F. Millero, RSMAS, Univ of Miami. The oceanic carbonate system (partial pressure of  $\text{CO}_2$ , pH and alkalinity) is changing rapidly due to an increased uptake of anthropogenic  $\text{CO}_2$  from the atmosphere. Current  $\text{CO}_2$  levels of ~380 ppm exceed pre-industrial levels of 280 ppm and have resulted in a reduction of oceanic pH by ~0.1. Furthermore,  $\text{CO}_2$  is expected to rise to levels possibly exceeding 1000 ppm by the year 2100 and ~1900 ppm by 2300, yielding oceanic pH reductions of 0.3-0.4 and 0.7-0.8 units, respectively. Placing this in perspective, the current rate of change in atmospheric  $\text{CO}_2$ , and thus the oceanic carbonate system, is ~100-times faster than what has occurred in the past 650,000 years, posing a challenge to marine organisms and ecosystems. The predicted acidification will result in a change in inorganic copper speciation, leading to more than a doubling and nearly a three-fold increase in the fraction of copper present as ionic  $\text{Cu}^{2+}$  by the years 2100 and 2200, respectively. From this speciation change alone, copper toxicity in seawater is expected to increase significantly. Elevated  $\text{CO}_2$  and ocean acidification challenge acid-base balance in marine organisms, a physiological parameter also affected by copper exposure. Thus, it is hypothesized that in addition to the direct effects of altered copper speciation, synergistic effects between exposure to elevated  $\text{CO}_2$ , reduced pH and substantially increased ionic copper will significantly increase copper toxicity. Ongoing embryo-larval toxicity tests on the marine pelagic fish cobia (*Rachycentron canadum*) aim at testing this hypothesis under current and predicted future  $\text{CO}_2$  and ocean acidification scenarios.

**WP132 Is Sulfur in Scallop Shells an Indicator of Stress Response in Ocean Acidification?** B. Broadaway, R. Hannigan, Univ Of Massachusetts Boston, Environmental, Earth, and Ocean Sciences. The burning of fossil fuels, and other natural processes, has led to an increase of carbon dioxide in the atmosphere. The equilibration of atmospheric carbon dioxide with surface waters causes the ocean pH to decrease from the current value of 8.1 through ocean acidification. Evidence suggests that as the ocean becomes more acidic, organisms such as the bay scallop, *Argopecten irradians*, will become more stressed due to increased energy demands to maintain shell deposition and growth. This stress, as hypothesized here, may cause an increase in the deposition of S-bearing organic macromolecules to aid in mineralization. There is also a potential for deposition of mineralized sulfate if the availability of carbonate species becomes limited. In the laboratory, *Argopecten irradians* were reared under air-carbon dioxide mixtures (pH = 8.0, 7.8, 7.5). Measurements of aquaria water quality (dissolved  $p\text{CO}_2$ , alkalinity, dissolved organic carbon, salinity, temperature, and pH) were measured or modeled. Fourier transform infrared spectroscopy was used to investigate the inorganic and organic shell matrix composition (pre- and post-treatment). Using principal component analysis, we identified changes in calcite, amide, and S-bearing macromolecule composition in shells of animals raised under acidic conditions. Impacts on growth and survival were also noted.

**WP133 Ocean Acidification in Estuaries: Interactive Effects of Temperature, Salinity and Elevated  $\text{P}_{\text{CO}_2}$  on Biomineralization and Metabolism of Oysters** I. Sokolova, A. Ivanina, Univ of North Carolina at Charlotte, Dept of Biology; G. Dickinson, E. Beniash, Univ of Pittsburgh, Dept of Oral Biology. Rising atmospheric  $\text{CO}_2$  levels lead to ocean acidification (OA), which can negatively impact calcifying organisms including mollusks. In estuaries, exposure to elevated  $\text{P}_{\text{CO}_2}$  often co-occurs with other stressors, such as fluctuating salinity and temperature, that can affect seawater chemistry, alter physiology of estuarine calcifiers and modify their response to OA. We studied the interactive effects of salinity or temperature combined with elevated  $\text{P}_{\text{CO}_2}$  on biomineralization and energy homeostasis in oysters *Crassostrea virginica*. In one experiment, juvenile oysters were exposed to one of two environmentally relevant salinities (30 or 15‰) either at current atmospheric  $\text{P}_{\text{CO}_2}$  (~400  $\mu\text{atm}$ , normocapnia) or  $\text{P}_{\text{CO}_2}$  projected by moderate IPCC scenarios for the year 2100 (~700-800  $\mu\text{atm}$ , hypercapnia). In a separate experiment, adult oysters were exposed to a combination of two temperatures (22 and 27°C) and  $\text{P}_{\text{CO}_2}$  (~400 and 800  $\mu\text{atm}$ ). We found that salinity and temperature modify physiological and biomineralization responses of oysters to hypercapnia. Exposure of juvenile oysters to elevated  $\text{P}_{\text{CO}_2}$  and/or low salinity led to a significant increase in mortality, depletion of tissue energy stores (glycogen and lipid) and negative soft tissue growth, indicating energy deficiency. However, tissue ATP levels were not affected by exposure to salinity and  $\text{P}_{\text{CO}_2}$  stress suggesting that the juveniles maintain

their cellular energy status at the expense of lipid and glycogen stores. In adults, elevated temperature alone or in combination with high  $P_{CO_2}$  did not affect respiration rate or tissue energy status. Elevated  $P_{CO_2}$  at 22 or 27°C led to transient oxidative stress in adult oysters indicated by accumulation of malondialdehyde- and 4-hydroxynonenal- protein conjugates. Exposure to hypercapnia combined with low salinity or elevated temperature stress negatively affected mechanical properties of oyster shells, resulting in reduced hardness and/or fracture resistance. Overall, our data show that juveniles are more sensitive to elevated  $P_{CO_2}$  than adults. Weakened shells and elevated basal energy demand in juveniles exposed to elevated  $P_{CO_2}$  (especially at low salinity) can reduce their protection against predators and other stresses, and divert metabolic resources from fitness-related functions such as growth and maturation. These changes are likely to negatively affect the survival of oyster populations in the face of OA. Supported by NSF award IOS-0951079.

#### WP134 Ocean Acidification Inhibits Navigation of Larval Orange

**Clownfish, *Amphiprion percula*** E. Wilcox Freeburg, Univ Of Massachusetts Boston; A. Rhyne, Roger Williams Univ; M. Tlusty, The New England Aquarium; R. Hannigan, Univ Of Massachusetts Boston, Environmental, Earth, and Ocean Sciences. Recent research into the impact of ocean acidification on marine organisms shows mixed responses in terms of calcification rate and behavioral endpoints. In particular, several studies on marine fish discuss potential susceptibility of specific fish species to olfactory sensory malfunction. Conflicting reports show earstone, or otolith, growth to be normal or accelerated. A combination of olfactory and gravitational ("hearing") cues is necessary for proper navigation and therefore survival. *Amphiprion percula*, orange clownfish, was tested for endpoint behavioral change in response to extant and predicted levels of  $pCO_2$ . We hypothesize that larval posture and post-settlement holding of station may be impacted under higher  $CO_2$  conditions due to impacts on the growing otolith. This hypothesis is supported by some evidence that larval otoliths grow more rapidly and are possibly dominated by vaterite when raised under low pH/high  $CO_2$  conditions. Future work will seek to elucidate the cause of the behavioral changes observed in this study.

#### WP135 Use of Caged Zebrafish Juveniles as Indicators of Pollutant Effect in Karstic Water Bodies

G. Rodriguez Fuentes, Centro para el Estudio del Agua, Unidad de Ciencias del Agua-CICY, Centro para el Estudio del Agua, Unidad de Ciencias del Agua-CICY, Centro para el Estudio del Agua; M. Soto, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología, Roger Williams Univ, Marine Biology. Yucatan Peninsula is a predominantly flat limestone region in the Southeast of Mexico. Bodies of surface water and streams are scarce or absent. Groundwater is the main source for human, industrial and agricultural consumption. Karstic areas in Yucatan are very permeable and are thus highly susceptible to aquifer contamination, some reports have already demonstrated groundwater contamination, but studies about organic pollutants are rare. A remarkable feature of the Peninsula, especially in the North, is the presence of cenotes that are water-filled sinkholes formed by the dissolution of limestone by carbonic acid. Cenotes have a high ecological value since they provide water and habitat for numerous species, some of them endemic. In this study we evaluated gene expression of vitellogenin (VTG) and cytochrome P-450 1A (CYP1A) in caged juvenile zebrafish. Cages were located in 13 different cenotes or aguadas (obstructed cenote), organisms were exposed during 15 days. Gene expression was evaluated using quantitative RT-PCR. Results indicated over expression of VTG gene in 7 water bodies. The highest relative expression was found in an "aguada" close to a cattle farm where VTG expression was more than 3000 times higher than expression found at controls. CYP1A over-expression was located in 3 water bodies, all of them located near villages or used for tourist activities. Monitoring of pollutants and biomarkers of effect should be done in these water bodies, in order to have a better understanding of the actual levels of pollutants that are present at Yucatan's aquifer.

#### WP136 Sensitivity of the Vitellogenin Assay: Ability to Discriminate Among Exposure Concentrations in Adult Fathead Minnows

R. Flick, USEPA, Office of Research and Development, USEPA, National Exposure Research Laboratory, USEPA, NERL; M. Crane, USEPA, National Exposure Research Laboratory; K. Jensen, USEPA, NHEERL; J. Oris, Miami Univ, Dept of Zoology. Vitellogenin is often used to infer exposure of an

organism to estrogenic substances. Vitellogenin gene induction and protein levels increase, up to a point, with concentration of estrogen and duration of exposure. A biomarker such as vitellogenin should exhibit sufficient sensitivity that it can discriminate among exposure concentrations to an extent greater than simple presence/absence of a contaminant. The goals of this study were to investigate the sensitivity of the vitellogenin assay and to determine if longer exposures increased sensitivity under controlled laboratory exposures. Fathead minnows were exposed to concentrations of ethinyl estradiol ranging from 0.05 to 100 ng/L for 48 hr, 96 hr and seven days in a flow-through diluter system. Vitellogenin gene expression was quantified in liver tissue and vitellogenin protein was quantified in plasma. Gene expression patterns were similar at 48 and 96 hr, but at 7 days expression was highly variable over many concentrations and the ability of the assay to discriminate among concentrations less than at the other two time points. Shorter exposures may provide greater sensitivity than longer exposures and are likely more tractable and cheaper to perform.

#### WP137 Application of a Long-Established Molecular Marker in Larval Teleosts to Evaluate Estrogenic Potential in Surface Waters and Wastewater Effluents

T.V. Reddy, US Environmental Protection Agency, National Exposure Research Laboratory; D. Lattier, US Environmental Protection Agency, Ecological Exposure Research Division; J.M. Lazorchak, D.A. Gordon, US Environmental Protection Agency, National Exposure Research Laboratory; M.E. Smith, The McConnell Group, Dynamac Corp., c/o United States Environmental Protection Agency. In recent years molecular indicators, diagnostic for exposure in aquatic systems, have been developed using teleostean models in laboratory and field settings. Our laboratory has previously shown that the gene for vitellogenin (VTG), a protein precursor of egg yolk in oviparous animals, can be induced to express in fathead minnow (FHM; *Pimephales promelas*) swim-up fry. FHM are widely distributed freshwater fish and fundamental among USEPA standard aquatic toxicology models. Early life stage (ELS) *P. promelas*, 48 hr post hatch and long-pec embryos, are currently being used as adult surrogates to measure transcription of Vtg, indicative for waterborne estrogen active compounds. The described ELS organisms were exposed to control (0), 0.625, 1.25, 2.5, and 5.0 ng L<sup>-1</sup> of the synthetic estrogen, 17 $\alpha$  ethynylestradiol (EE2). Following exposure, total RNA was isolated from single larvae in addition to statistically requisite numbers of groups comprising pooled larvae in quantities of 5 and 10. Synthetic oligonucleotide primers, specific for *P. promelas* VtgI transcripts, were used to thermally amplify RNA by way of QPCR. Analyses of data obtained thus far, suggests that use of ELS bioindicators affords a viable approach to detect estrogenic EDCs. Having yet to analyze RNA representing the range of exposure concentrations, the level of Vtg detection in FHM larvae (5.0 ng L<sup>-1</sup>) suggests that results are equal to values obtained from QPCR amplification of liver-specific RNA from adult FHM exposed to the same concentration of EE2 for 48 hrs. Although not a specific aim in development of this method, we nevertheless note that EE2 induced expression of FHM Vtg was measurable in single larva. The rationale for our bioindicator design is manifold. Use of larval organisms significantly reduces biomass, which is reflected in scale of exposure; achieved by means of fewer resources with substantial cost savings in culturing, labor, and exposure volumes. Larval fish are nevertheless regarded as 'whole animals'; however, experimental procedures carried out with limited ELS biomass, is a step toward future Agency compliance, and likely to be perceived as more palatable by those advocating for animal welfare. In addition to considerable cost and time savings, elimination of animal necropsy is a welcome facet of this protocol. This approach provides a simple, economical and transferable monitoring routine for surface waters and WWTTP effluents.

#### WP138 Metallothionein and Vitellogenin Genes of Zacco Platypus and Their Biomarker Potential for Aquatic Contaminants

S. Lee, Seoul National Univ, School of Public Health; S. Kim, Seoul National Univ, School of Public Health, Room # 736, Bldg # 220.; J. Kim, Korea Advanced Institute of Science and Technology; W. Kim, J. Kim, Korea Institute of Toxicology; H. Shin, C. Choi, Korea Maritime Univ; S. Lee, Korea Institute of Toxicology; K. Choi, Seoul National Univ, School of Public Health. *Z. platypus*, a freshwater teleost, is widely distributed in Korea and eastern Asia, and is considered as a candidate species that can be used as an indicator reflecting the regional aquatic ecology. The need for developing sensitive biomarkers that may reflect adverse impacts of chemical exposure is present for *Z. platypus*. In this study, we employed *Z. platypus* as a potential indicator species, and evaluated *metallothionein* (MT) and *vitellogenin* (VTG)



genes for the usefulness as biomarkers of exposure to metals and endocrine disrupting chemicals. For this purpose, we carried out phylogenetic study with cDNA sequences of both genes of *Z. platypus*. For phylogenetic analysis, BlastX searching was conducted to compare amino acid sequences and ClustalX, BioEdit and Mega 4 software were used to construct phylogenetic tree. Quantitative PCR analysis was conducted after the 14 d exposure to cadmium (Cd) and bisphenol A (BPA), and responses of both genes were measured. After exposure liver tissue of three individual *Z. platypus* were pooled for the quantitative PCR analysis. The phylogenetic analysis of cDNA sequences of both genes demonstrated high conformity with the genes of other teleost species, suggesting similar functions of these genes in this indigenous fish. *MT* and *VTG* mRNA expressions showed positive linear relationship with Cd and BPA exposures. The mRNA expression of *MT* showed significant dose-dependent positive trend in both male and female after Cd exposure ( $p$  for trend = 0.012 in male, and 0.013 in female). *VTG* mRNA expression also showed positive linear trend after BPA exposure ( $p$  for trend = 0.004 in female, and  $p$  for trend = 0.112 in male). Both genes were determined to be useful biomarkers of metal and endocrine disrupting chemicals exposure in *Z. platypus*.

**WP139 DNA Damage in Cichlid Fish and Environmental Forensic Analysis of the Origin of PAHs at a Guatemalan Oil Field** C. Theodorakis, Southern Illinois Univ Edwardsville, Dept of Biological Sciences and Environmental Sciences Program, Southern Illinois Univ Edwardsville, Environmental Sciences; J. Bickham, Purdue Univ, Center for the Environment; K.C. Donnelly, Texas A&M Univ, Dept of Veterinary Anatomy and Public Health; T. McDonald, Texas A&M Univ, Dept of Civil Engineering. This study focused on several wetlands in Laguna del Tigre National Park (Guatemala) as part of Conservation International's Rapid Assessment Program. Sediment and water samples were collected from a lagoon near Xan field, Guatemala's largest oil facility, and 3 other sites for determination of levels of polycyclic aromatic hydrocarbons (PAHs). Cichlid fish (*Thorichthys meeki* and *Vieja synspila*) were collected for determination of DNA strand breakage (by gel electrophoresis) and chromosomal breakage (flow cytometry). PAHs in water and sediment were extracted with methylene chloride and analyzed by GC/MS. Environmental forensic analysis was also carried out using three metrics to draw inferences about the origin (pyrogenic, petrogenic, diagenic) of the PAHs in the sediment. These metrics included a) concentration (mg/kg) of PAHs with 4 or more rings, b) a "pyrogenic index" defined by as  $\sum(\text{all other 3-6 ring PAHs})/\sum(\text{alkylated [naphthalenes, phenanthrenes, dibenzothiophenes, fluorenes, and chrysenes]})$ , and c) ratio of chrysene/phenanthrene in sediments. Multivariate analyses were also used to analyze all three of these variates together. For *T. meeki* from Xan field, chromosomal breakage and strand breakage was greater than in at least two of the three reference sites. For *V. synspila*, chromosomal breakage and strand breakage were greater in Xan than one of the two reference sites. Patterns of aqueous PAH concentrations, suggests that fish are affected by anthropogenic contaminants. PAHs were elevated at some reference sites, but environmental forensic analysis suggested a pyrogenic or diagenic origin. The environmental forensic analysis also suggested that PAHs near the oil well injection site were primarily of petrogenic origin, and the ratio of petrogenic:pyrogenic PAHs decreased with distance from the oil field. The data are consistent with the hypothesis that oil field brines injected into the ground water caused genotoxicity in fish at Xan field, and it is also possible that pyrogenic PAHs influence levels of DNA damage in reference sites. These analyses represent one of the first efforts to examine genotoxicity in native Mesoamerican cichlids.

**WP140 Evidence that Oil Droplets Contribute Only to a Limited Extent to the Overall Toxicity of Oil Dispersion to Larvae of Atlantic Cod (*Gadus morhua*)** B. Hansen, SINTEF Materials & Chemistry, Marine Environmental Technology; P. Olsvik, NIFES; T.R. Storseth, SINTEF Fisheries and Aquaculture; D. Altin, BioTrix; A.J. Olsen, Norwegian Univ of Science and Technology, Biology; I. Overrein, SINTEF Fisheries and Aquaculture; F. De Laender, Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent Univ; T. Nordtug, SINTEF Materials and Chemistry, Marine Environmental Technology. Oil exploration in the Atlantic moves towards spawning and nursery areas of fish species that sustain some of the world's largest fishery industries. Numerical models are needed to predict the fate of accidentally oil spilled and its toxic effect on local biota. Toxicity is expected to be caused by the water soluble fraction of dispersed oil, however, little is known about the possible effects of oil droplets. A custom made exposure

rig was built for assessing the effects of oil droplets, and first feeding cod larvae were exposed for four days to either dispersed oil (with oil droplets) or the corresponding filtered dispersion (the water soluble fraction [WSF]) at six concentrations. The endpoints analysed for ranged from molecular (transcriptomics and metabolomics) to fitness-related (survival probability and assimilation rate). A concentration-dependent decrease in survival probability was observed following exposure to dispersed oil with EC50 of about 40 µg/L ΣPAH. Microarray analyses revealed that oil exposure affects drug metabolism, endocrine regulation, cell differentiation and proliferation, apoptosis, fatty acid biosynthesis and tissue development. A set of differentially regulated transcripts encoding proteins involved in bone development suggests that oil exposure may negatively affect skeletal system development. These observations were supported by a concentration dependent decrease in growth and assimilation rate with EC50 of about 2 µg/L ΣPAH. In conclusion, the results from this experiment indicate that numerical models that explicitly consider oil droplet toxicity – in addition to the toxicity of the WSF – on the examined endpoints are therefore unlikely to be more accurate than models that neglect oil droplet toxicity.

**WP141 Enhanced Fluorescent Protein Transgenic *Daphnia magna* and Their Applications** T. Lee, Chonbuk National Univ, Dept of Bioprocessing Engineering; S. Lee, Korea Research Institute of Chemical Technology, Toxicology Research Center; Y. Kim, Chungbuk National Univ, School of Life Science; J. Min, Chonbuk National Univ, Dept of Bioprocess Engineering. A transgenic water flea, *Daphnia magna*, able to express a green fluorescent protein has been created to establish a novel system for a rapid detection of environmental stress. A 32bp DNA fragment containing the 18s-ribosome constitute promoter of *Daphnia* was generated by invitro annealing the commercial primers at 80°C. This *Daphnia* 18s-ribosome DNA (D18s) was inserted and replaced the CMV promoter fragment that was located ahead to the gfp gene in the EGFP vector (Enhanced Green Fluorescent Protein). The successful recombinant plasmid confirmed by the DNA sequencing was introduced into the earlier developing-state *Daphnia* eggs (round shape) using microinjection technique. After 48h incubation in the 20°C chamber, the injected eggs able to develop to juveniles were observed at the 488nm wavelength by the confocal fluorescent microscope to confirm the presence of green fluorescent protein. Our results show that the green fluorescent protein was substantially found in the upper part of the transgenic organisms (e.g., head, back) while no fluorescent signal was detected in the control organism. It demonstrated that a transgenic *D. magna* expressing green fluorescent protein was firstly generated and would be promising to build a novel testing system for easily monitoring environmental stress.

**WP142 Identification and Characterization of an Superoxide Dismutase Genes (SOD) in *Chironomus riparius* Exposed to Environmental Pollutants** S. Park, P.M. Nair, Univ of Seoul; J. Choi, Univ of Seoul, School of Environmental Engineering. Superoxide dismutase (SOD, EC 1.15.1.1) represents one kind of enzyme involved in scavenging the high level of reactive oxygen species (ROS) into molecular oxygen and hydrogen peroxide. In this study, a copper/zinc superoxide dismutase (Cu/ZnSOD) gene and a manganese superoxide dismutase (MnSOD) gene of *Chironomus riparius* (CrSODs) was identified from Expressed Sequence Tags (ESTs) database generated using pyrosequencing. A multiple sequence alignment of C. riparius sequences revealed high homology with other insect sequences the amino acid level. Phylogenetic analysis of CrSODs grouped it with SODs of other insect taxa. CrSOD genes expression was analyzed during different in response to treatment with various concentrations of environmental stresses for variety of time interval. Cadmium dichloride (CdCl<sub>2</sub>) as heavy metal, nonylphenol (NP) as endocrine disrupting chemical (EDCs), silver nanoparticles (AgNPs) as nano material were used as stressors. These results show that SOD enzyme have important role and its potential as a biomarker to various environmental pollutants. Keywords : *C. riparius*, Superoxide dismutase (SOD) This work was supported by Mid-career Researcher Program through NRF grant funded by the MEST(2010-0027722)

**WP143 Characterization of a cDNA of CYP1A1 and its Modulation by Environmental Xenobiotics in Fourth Instar Larvae of *Chironomus riparius*** P.M. Nair, S. Park, G. Chung, Univ of Seoul; J. Choi, Univ of Seoul, School of Environmental Engineering. Modulation of Cytochrome P450 super family of genes by environmental xenobiotics is used as a potential biomarker in ecotoxicological studies. They are highly inducible at the



transcript and enzyme level by a variety of environmental pollutants. A 1978 bp CYP1A1 cDNA was identified by screening through a ESTs data base developed from the fourth instar larvae of *Chironomus riparius* using 454 pyrosequencing. The CYP1A1 cDNA included an open reading frame (ORF) of 1587 base pairs (bp) encoding a putative protein of a 528 amino acid protein. There was a 267 bp 5' and a 123 bp 3' untranslated region with a polyadenylation signal site (AATAAA). Alignment of the deduced amino acids of *C. riparius* CYP1A1 cDNA showed high level of sequence similarity to homologous CYP1A1 from other species and the presence of conserved amino acids. *CrCYP1A1* had a putative heme-binding cysteine at position 471 in the typical P450 signature sequence of 463- FGIGPRNCIG-473. The phylogenetic relationships of CYP1A1s among 13 fish species were analyzed by a distance method. The CYP1A1 transcript was present at all life stages. Modulation in the transcript level of CYP1A1 gene was observed after exposure to different environmental pollutants and the results are discussed. Key words: *C. riparius*; CYP1A1; Cadmium; Benzo[a]pyrene; BPA; Nonylphenol; Chlorpyrifos This work was supported by Mid-career Researcher Program through NRF grant funded by the MEST(2010-0027722)

**WP144 Chitobiase Activity as a Measure of Recovery in *Daphnia magna*: Application to Pulsed Exposure to Glyphosate and its Formulations** J.L. Rodriguez Gil, Guelph Univ, School of Environmental Sciences, Univ of Guelph, School of Environmental Sciences; K. Solomon, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Centre for Toxicology, School of Environmental Sciences; M. Hanson, Univ of Manitoba, Dept of Environment and Geography, Univ of Manitoba, Faculty of Environment; R. Prosser, Univ of Guelph, School of Environmental Sciences. Chitobiase is a chitinolytic enzyme released into aquatic systems by zooplankton during ecdysis. Previous research has shown that the activity of chitobiase in the surrounding media is positively correlated with growth in various species of freshwater and marine zooplankton. Therefore, chitobiase shows potential as measure of recovery after exposure to stressors that inhibit growth. The aim of this study was to evaluate whether chitobiase activity in the surrounding media of pre-exposed *Daphnia magna* individuals could provide similar or, preferably, better results than conventional predictors of recovery, such as time to first brood or number of neonates, as this could make the assay more rapid and cost-effective. In this study, *D. magna* were exposed for 48 hr to a range of concentrations of glyphosate, the surfactant MON 0818, and a glyphosate formulation containing the surfactant. Following exposure, individual *Daphnia* were transferred into clean reconstituted water and the activity of chitobiase released by each individual was measured every 48h for a total 14 days. Time to first brood and the number of neonates produced in the first brood were also determined during the recovery period. Chitobiase activity exhibited a hormetic response during the 96h after transfer, with greater activities observed for intermediate concentrations. Complete recovery, defined as the lack of significant differences from the control on any of the studied parameters occurred in all tested concentrations of MON0818. Chitobiase activity, number of neonates in the first brood, as well as time to first brood, were significantly lower in the daphnids exposed to high concentrations of glyphosate or the tested commercial formulation as compared to MON0818 alone. The results indicate that activity of chitobiase may be an effective measure of recovery for *D. magna* and possibly other freshwater arthropods.

**WP145 Characterization of Controlled Release Formulations of Novaluron** P. McManus, J. Serey, L. Veldhuis, J. Hall, Univ of Guelph, school of environmental science. Mosquitoes pose a serious risk to public health around the globe as vectors of human pathogens such as the West Nile virus. Since 2002, about 34000 cases of West Nile viral infection in humans have been reported in Canada and the USA, resulting in hospitalizations and deaths. Consequently, it is extremely important to control mosquito populations especially at their breeding sites, i.e., standing water systems. A number of pesticides including novaluron, a member of benzoylphenyl urea family, are effective at controlling mosquitoes. Two new slow-release, wax-based formulations containing novaluron have been developed to regulate the release of the active ingredient in standing water. The goal of this research is to determine how pH, salinity, water hardness, organic matter and temperature affect the release and efficacy of novaluron. Of the parameters tested, only temperature affected the rate of release of novaluron from the formulations, while only organic matter reduced the concentration of novaluron once it partitioned into water. The slow release formulation provided effective mosquito control for at least 6 months

making these formulations more effective than the EC formulation for long-term mosquito control.

**WP146 Early Life Stage Toxicity of Select Pharmaceuticals to the Fathead Minnow, *Pimephales promelas*** M.D. Overturf, C. Overturf, Univ of North Texas, Dept of Biological Sciences; L.A. Constantine, Pfizer Global Research and Development, Pharmacokinetics, Dynamics & Metabolism, Pfizer, Inc., Pharmacokinetics, Dynamics and Metabolism; D.B. Huggett, Univ of North Texas, Dept of Biological Sciences. Human pharmaceuticals are routinely being detected in the environment and there is growing concern whether these drugs could elicit effects on aquatic organisms. Regulatory paradigms have shifted accordingly, with a greater emphasis on chronic toxicity data as opposed to acute data. The OECD 210 Early Life Stage Test has been proposed as a good measure of the potential for pharmaceuticals to elicit chronic toxicity. To begin building a dataset regarding the early life stage toxicity of pharmaceuticals to fish, fathead minnows (FHM) were exposed to amiodarone, carbamazepine, clozapine, dexamethasone and fenofibrate. Survival and growth were used to assess chronic toxicity in FHM at 28 days post-hatch. Exposure of FHM to carbamazepine and fenofibrate resulted in no significant adverse effects at the concentrations tested. Dexamethasone exposed FHM showed a significant decrease in survival at a concentration of 576.8 mg/L; however, growth was not impacted at the concentration tested. Exposure to amiodarone and clozapine resulted in a significant decrease in survival and a significant increase in growth at concentrations of 1021.1 and 30.8 mg/L, respectively. As the effect levels derived in this study are above concentrations observed in the environment, these data suggest that these pharmaceuticals present a low risk to larval fishes.

**WP147 Ecotoxicity of Sediments Sampled in Urban Rivers and Streams Dominated by Domestic Sewage: Potential Contributions of PPCPs** Y. Yasuda, J. Morita, I. Tamura, Univ of Tokushima; N. Nakada, M. Narumiya, S. Hanamoto, Kyoto Univ; Y. Kameda, Saitama Institute of Environmental Sciences; K. Kimura, Saitama City Institute of Health Science & Research; H. Yamamoto, The Univ of Tokushima, Faculty of Integrated Art & Science. We have investigated the aqueous concentrations of PPCPs and their possible contributions on whole toxicity of the water samples collected in urban rivers and streams in Tokushima, Kyoto, and Saitama, Japan, dominated by treated or untreated domestic sewage. Combining the whole toxicity tests of the water samples, detected concentrations of PPCPs, toxicity tests for individual PPCPs using green algae (*Pseudokirchneriella subcapitata*), cladoceran (*Ceriodaphnia dubia*) and fish (*Danio rerio*), slight contribution on the cladoceran reproduction or survival and moderate to relatively high contribution on algal growth were observed. The potentially strong contributors among the PPCPs include antimicrobials such as triclosan and triclocarban. In present study, we sampled both water and sediment at the sites we previously examined the aqueous samples, and tested the sub-chronic toxicity on benthic organisms (*Chironomus yoshimatsui* and *Hyalella azteca*). As results of the whole sediment toxicity, we found slight toxicities for the organisms. We also conducted toxicity tests for individual PPCPs, in particular those with relatively higher hydrophobicity and detected concentrations, such as triclosan, oxybenzone, and octylmethoxycinnamate. The contribution of these compounds are limited most of the case, and some other compounds such as heavy metals and PAHs are the possible main contributors of whole sediment toxicity. Results of our study could contribute the possible prioritization of PPCPs to be further investigated.

**WP148 Applicability of Standard Antibiotic Toxicity Tests to the Ambient Aquatic Environment** F.L. Hellweger, Northeastern Univ, Northeastern Univ, Civil & Environmental Engineering; X. Ruan, E. Cherchia, S. Sanchez, Northeastern Univ. The dissolved organic matter (DOM) concentration in conventional minimum inhibitory concentration (MIC) laboratory assays is typically three orders of magnitude higher than in the ambient aquatic environment. Partitioning of the antibiotic to DOM could affect bioavailability or potency. This question was investigated using laboratory experiments with *E. coli*, tetracycline and DOM varied over six orders of magnitude, which resulted in the same toxicity endpoint of 1 mg/L.

**WP149 Do Antidepressant Mixtures Have Additive Effects on Fish Predatory Behavior?** J.H. Bisesi, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX); K. Johnson, Southern Illinois Univ at

Edwardsville, Dept of Chemistry & Environ. Sciences; S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology (ENTOX) The concentrations of antidepressants detected in the effluent and receiving streams of wastewater treatment plants have been shown to be relatively non-toxic in traditional toxicity tests. But the neurochemical mode of action of antidepressants warrants investigation of the effects these chemicals may have on fish behavior. Using a predator prey bioassay previously designed in our lab, we have shown that the antidepressants fluoxetine (Prozac®) and venlafaxine (Effexor®) individually increase the time it takes for a predator to capture its prey. Concentrations that cause this effect are typically 1-2 orders of magnitude less than LC<sub>50</sub>'s from traditional toxicity tests, but are still 1-2 orders of magnitude higher than those found in wastewater treatment plant effluent. In the environment these antidepressants would be found in complex mixtures with other antidepressants, as well as other chemicals. Since the effects of mixtures are not well understood the objective of the current study was to characterize the behavioral effects of a simple mixture of the two antidepressants we had previously investigated individually, fluoxetine and venlafaxine. An effect level was selected to represent one toxic unit for each of the chemicals. Levels of 0.5, 1, and 2 toxic units were tested to determine how these chemicals may interact to cause effects on fish predatory behavior. In addition to behavioral endpoints, fish brains were collected throughout exposures and analyzed for monoamine profiles. Previous work has shown that antidepressant exposure decreases brain serotonin, which can be correlated with an increase in time to capture prey. The current study will determine how multiple antidepressants interact to alter brain chemistry, and whether this interaction can cause an increased/decreased effect on fish predation behavior. Initial results indicate that fluoxetine and venlafaxine may interact in an additive manner.

**WP150 Effects of Pharmaceuticals in Environment on Aquatic Organisms** N. Tatarazako, National Institute for Environmental Studies, Center for Environmental Risk Research, National Institute for Environmental Studies, Endocrine Disrupter Research Laboratory; T. Oka, H. Watanabe, National Institute for Environmental Studies, Research Center for Environmental Risk; T. Suzuki, Tokyo Metropolitan Institute of Public Health; T. Nishimura, National Institute of Health Sciences, Division of Environmental Chemistry. It is concerned that environmental pharmaceuticals have on an aquatic organism recently. In our study, seven chemicals (diclofenac sodium, mefenamic acid, carbamazepine, fenofibrate, crotamiton, epinastine hydrochloride, and fumagillin) were investigated. These substances except fumagillin were detected from the Tama River (Japan), which is main river in the arcuate line between Tokyo and Kanagawa. Bioassays were carried out according to three traditional toxicity tests (algae; *Pseudokirchneriella subcapitata* growth inhibition, *Daphnia*; *Ceriodaphnia dubia* reproduction, and fish; *Danio rerio* short-term toxicity test on embryo and sac-fry stages). Each pharmaceutical showed different effects among three species. Diclofenac sodium showed weak toxicity in *Daphnia* (NOEC 2.0 mg/L) and fish (NOEC 2.5 mg/L). Carbamazepine showed weak toxicity in algae (NOEC 8.6 mg/L) and *Daphnia* (NOEC 21 mg/L). Crotamiton showed weak toxicity in algae (NOEC 5.2 mg/L) and *Daphnia* (NOEC 6.2 mg/L). Mefenamic acid showed slightly strong toxicity in algae (NOEC < 0.3 mg/L) and fish (NOEC 1.3 mg/L). Epinastine showed slightly strong toxicity in algae (NOEC 0.6 mg/L) and *Daphnia* (NOEC 2.8 mg/L). The effect of fenofibrate was not shown with all species. Fumagillin showed very strong toxicity for reproduction of *C. dubia* (NOEC 0.03 mg/L). Fumagillin is one of the anticancer drugs used as angiogenic inhibitor to a cancer cell. It is very interesting to have a strong effect for the *Daphnia* which there is not a blood vessel. Our results showed that various pharmaceutical which varied in effects had for wildlife. However, it was shown that the effects mechanism for the wildlife might be different from the effects mechanism for humans of the pharmaceutical. It is thought that it is necessary to pay its attention to the different endpoint conventionally to evaluate environmental effect of the pharmaceuticals.

**WP151 Investigation of the In Vitro Metabolism of Diazepam in Channel Catfish (*Ictalurus punctatus*)** C. Overturf, D.B. Huggett, Univ of North Texas, Dept of Biological Sciences. Many pharmaceuticals have been detected in surface waters over the past few years, which may pose adverse effects to aquatic life. Thus, the following study examined the in vitro metabolism of diazepam, a model benzodiazepine, in microsomal fractions of the liver from channel catfish. The loss of parent compound (diazepam),

as well as the generation of biologically active metabolites (nordiazepam, temazepam and oxazepam) was investigated. An NADPH regeneration system was used to simulate phase I reactions and 100µM diazepam was added to the system with or without the presence of 10µM of a cytochrome P450 inhibitor. Samples were taken at selected time points and placed in methanol to stop any further reaction. The samples were then processed and analyzed using LC-MS/MS. α-naphthoflavone (ANF), 17α-ethinylestradiol (EE2) and lauric acid were used as specific inhibitors of CYP1A, CYP3A27 and CYP2K1, respectively; whereas ketoconazole was used as a non-specific cytochrome P450 inhibitor. ANF resulted in no significant inhibition of diazepam or the formation of metabolites. EE2 resulted in complete inhibition of diazepam and 57% inhibition of temazepam at 60 minutes; however, did not alter nordiazepam formation. Lauric acid inhibited diazepam by 87% at 60 minutes, but did not alter metabolite formation. Ketoconazole did not significantly inhibit diazepam, but inhibited the formation of nordiazepam and temazepam by 69% and 97% at 60 minutes, respectively. Thus, it appears the CYP1A may not be involved in the metabolic pathway of diazepam in catfish. The resulting inhibition of diazepam by EE2 and lauric acid indicates that CYP3A27 and CYP2K1 may be the primary pathways that diazepam is metabolized. Additionally, EE2 inhibited the formation of temazepam, which indicates that the formation of temazepam may also be mediated through CYP3A27. The formation of nordiazepam was only inhibited by ketoconazole, indicating that its formation is mediated through cytochrome P450, but not any of the specific isoforms investigated. Thus, the inhibition of diazepam metabolism via cytochrome P450 inhibition could lead to increased concentrations of this compound. Therefore, it is recommended that co-exposure bioconcentration studies should be conducted to determine the potential to modulate the bioconcentration of diazepam.

**WP152 The Change of Energy Relative Biomarkers of *Daphnia magna* Exposed to Tetracycline and its relationship to Organismal Responses in Multi-Generations** H. Kim, Gwangju Institute of Science & Technology, Dept of Environmental Science and Engineering, Gwangju Institute of Science & Technology, School of environmental science and engineering; S. Yu, M. Lee, Korea Atomic Energy Research Institute; T. Kim, Gwangju Institute of Science and Technology, School of Environmental Science and Engineering; S. Kim, Gwangju Institute of Science & Technology, School of Environmental Science and Engineering. The objectives of this study were to estimate the effect of tetracycline on energy relative biomarkers such as available energy contents and energy consumption and to link the biochemical indicators in the cellular level with the organismal level effect. The target compound was continuously exposed to daphnids over four successive generations at the sublethal concentrations including 0.1-10.0 mg/L. The biochemical endpoints such as available energy contents and energy consumption were measured to evaluate the effect of toxicant-induced stress on the metabolic balance of test organism. Energy reserve was calculated by measuring total protein, glycogen and lipid contents and energy consumption was estimated by measuring the activity of electron transport system (ETS). As a result, the contents of energy reserving compounds showed the concentration-dependent decrease in all generations. The concentration of protein, glycogen, and lipid in control was 32.4±4.5, 0.722±0.113, and 14.40±0.52 ug/organism, respectively. The content of each component was decreased by 25.4, 62.2, and 40.1% after 10 mg/L of exposure at parent generation. However, the rate of reduction was changed with increasing generation number. For example, the rate of glycogen reduction exposed to 10 mg/L was changed to 62.2% (F0), 61.5% (F1), 37.9% (F2), and 33.1% (F3). It suggests that the energy related biomarkers have a significant relationship with organismal effects such as growth and reproduction. Also, there is a tendency of energy related biomarkers with number of generation. It seems that trade-off mechanism might exist between the somatic growth and reproduction. Moreover, the energy content and expenditure might contribute to the organismal effect of *D. magna*, resulting in the change of population.

**WP153 The Effects of Bupropion on Predation Behavior and Brain Chemistry in Hybrid Striped Bass** L. Sweet, Clemson Univ, Institute of Environmental Toxicology; J.H. Bisesi, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX); S.J. Klaine, Clemson Univ, Institute of Environmental Toxicology (CU-ENTOX), Clemson Univ, Institute of Environmental Toxicology (ENTOX) The widespread use of pharmaceuticals has led to increased detection of these chemicals in wastewater effluents and receiving streams. Many of these pharmaceuticals are antidepressants,

intended to alter brain chemistry in humans; therefore, aquatic organisms with similar biochemical pathways may be vulnerable to the presence of these drugs in their environment. A bioassay has previously been developed in our lab to quantify the effects of antidepressants on predation behavior in hybrid striped bass (*Morone saxatilis* x *M. chrysops*) through measuring the time to capture prey (*Pimephales promelas*). Previous studies have shown that fluoxetine, a selective serotonin reuptake inhibitor, elicited an increased time to capture prey and a decrease in brain serotonin levels. Moreover, upon exposure to venlafaxine, a serotonin and norepinephrine inhibitor, an increased time to capture prey has also been observed. Therefore, by examining bupropion, a norepinephrine and dopamine reuptake inhibitor, we hope to elucidate the role of specific monoamines on brain chemistry and behavior. This information may be used to generate a model to evaluate the effects of antidepressants on fish predation behavior based on the targeted monoamines of the drug.

#### WP154 Uptake of Pharmaceuticals from Soils into Earthworms

(*Eisinea fetida*) L. Carter, Univ of York, Environment Dept; J. Ryan, GlaxoSmithKline; A. Boxall, Univ of York, Environment Dept. Pharmaceutical compounds are increasingly being detected in soils due to the application of sewage sludge and use of reclaimed wastewater for irrigation. Once in soil, pharmaceuticals can be taken up by soil dwelling organisms. Accumulated pharmaceuticals may then affect the health of the organisms or be transferred up through food chains. This study therefore investigated the uptake of two pharmaceuticals, fluoxetine and diclofenac, into earthworms (*Eisinea fetida*). Diclofenac is an acidic pharmaceutical whereas fluoxetine is a basic compound. The studies were based on the OECD Guideline 317 and involved a 21 day uptake phase followed by a 21 depuration phase. Studies were performed using radiolabelled compounds to allow for a lower limit of detection thus enabling environmentally relevant concentrations of both compounds to be used in the study. A continual increase in uptake of both fluoxetine and diclofenac into worms was observed over 21 days. At the end of the uptake phase, concentrations of fluoxetine had reached 0.028 mg/kg and diclofenac had reached 0.032mg/kg. When exposed worms were transferred to clean soil, both compounds were found to depurate rapidly with only trace amounts being detected in the worms after 21 d depuration. Parallel studies, using unlabelled compounds, were run alongside the radiolabelled uptake studies to determine the extent of degradation of the parent compounds. Further Radio-HPLC investigation was also able to determine that the radioactivity detected in the earthworm and soils was that of the parent compound and not of a metabolite. This study is one of the first to explore the uptake and depuration of pharmaceuticals, commonly found in the terrestrial environment, into earthworms. Future work includes investigation into how soil properties can influence uptake. Studies are also planned to assess the uptake of pharmaceuticals from soils into plants.

#### WP155 Evaluating Environmental Persistence Screen

C.A. Smith, Smithers Viscient, Senior Consultant; D. Sacker, S. Erler, Smithers Viscient; R. Hannah, GlaxoSmithKline, CEHS – RS 1105 To support product stewardship activities, environmental hazard classification and communication, and development of transportation criteria, Persistence, Biodegradation and Toxicity assessments are required. The scope of the above responsibilities involves developing environmental impact studies (environmental toxicology and environmental fate), ecological risk assessment and risk reduction strategies. As part of this, persistence evaluations utilizing biodegradation methods (OECD 301, 302 303 and 314 ) are used to determine ready and inherent biodegradation, mass balance and 1<sup>st</sup> order kinetic data. These studies are time consuming (minimum 28 days) and costly. They are run late in development and are not suitable to be run for screening purposes earlier in the development process when environmental issues could be potentially avoided. The purpose of this project is to design and implement a higher through-put, smaller scale wastewater biodegradation screening assay to assess chemical persistence in the Waste Water Treatment Facilities (WWTFs) that could be applied early in product selection and development activities to assess hazard and risk. These data along with other physical and environmental properties would be critical in developing: a PBT Profile – to facilitate early stage environmental hazard categorization. Environmental Fact Sheet–Material Safety Data Sheet – to support waste minimization and mitigation during prelaunch activities. Support development of an effective environmental testing strategy which focuses on highest intrinsic hazards and is critical to compound registration and marketing authorizations.

Development of product lifecycle environmental risk profiles and site-specific manufacturing risk assessments.

#### WP156 Human Health and Pharmaceuticals in the Environment: A Review of Existing Practice

E.S. Williams, Baylor Univ, Baylor Univ. Recent concerns over potential human and ecological risks arising from pharmaceuticals in the environment have spurred significant effort in the scientific community. The potential for adverse health outcomes associated with these substances has implications for wastewater treatment, water reuse, practices in solid and liquid waste disposal, and manufacturing. A relatively small number of human health risk assessments for pharmaceuticals in the environment have been published to date. These documents often address a small number of individual pharmaceutical substances, with carbamazepine, clofibrate acid, cyclophosphamide, sulfamethoxazole, and trimethoprim being more frequent targets of study. These exercises focus on ingestion of water and fish as routes of exposure, and employ a variety of methods to assess magnitude of these potential exposures, assign safety values, and characterize risk. With a few exceptions, the risks of adverse health outcomes resulting from exposure to pharmaceuticals have been judged to be low. This presentation will summarize the methodologies used in these published exercises, and discuss the associated uncertainties.

#### WP157 Ketoconazole Increases the Endocrine Disruption Potential of Ibuprofen Exposure in the H295R Cells and Japanese Medaka

K. Ji, Seoul National Univ, School of Public Health; K. Choi, Seoul National Univ, School of Public Health, Seoul National Univ, Dept of Environ. Health; K. Kwak, School of Public Health, Seoul National Univ; J. Kim, Korea Univ, Division of Environmental Science and Ecological Engineering; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; X. Zhang, Toxicology centre, Univ of Saskatchewan, State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing Univ, School of the Environment. Pharmaceuticals residues found in the aquatic environment usually occur as mixtures, while most of toxicity tests were made on individual chemicals. In the present study, we investigated whether exposure to low level ketoconazole increases the estrogenicity of ibuprofen exposure. Ibuprofen was reported to increase estrogenicity in fish. After 48 hr exposure to ibuprofen alone or in combination with ketoconazole, the production of 17 $\beta$ -estradiol (E2) and testosterone (T), the activity of aromatase, and the mRNA expression of steroidogenic genes and CYP2C9 gene were measured. In addition, concentrations of E2 and T in blood plasma, and the mRNA expression of two steroidogenic genes (CYP17 and CYP19b) were also determined in male Japanese medaka fish after 14 d exposure. Ibuprofen alone increased E2 production, and enhanced both aromatase activity and transcription of CYP17, CYP19 or CYP11 $\beta$ 2 genes in the cells, which are important components of steroidogenic pathways. In medaka fish, while ibuprofen only exposure at 0.2 mg/L did not cause any significant effects on E2 concentration compared to that of control, E2 production was more elevated in combination with ketoconazole, i.e., up to 1.5-fold compared to ibuprofen only exposure. In addition, a combined exposure to ibuprofen and ketoconazole resulted in an elevated expression of CYP17 and CYP19b mRNAs in male medaka, compared to ibuprofen only exposure. This study indicates that non-effective concentrations of ketoconazole can increase the potential for endocrine disrupting effects of ibuprofen in both human adrenal cell line and the freshwater fish.

#### WP158 The Ecotoxicity of Photocatalytic Nano-TiO<sub>2</sub> Particles on

Bacteria Exemplified by E. coli and B. subtilis A. Erdem, Akdeniz Univ, Environmental Engineering; C. Huang, Univ of Delaware, Civil & Environmental Engineering. Ecotoxicological effects of nanoparticles (NP) are still poorly documented while their commercialization increases in many production and manufacturing sectors. Among the various NPs, TiO<sub>2</sub> has being the most used nanomaterials in industry; from wall paints to cosmetic products and from textile products to children toys. Besides their advantages and they are regarded as a biocompatible material in the absence of photoactivation, nano-TiO<sub>2</sub> have also been shown to exhibit strong cytotoxicity when exposed to UV and solar irradiation. In order to understand the bacterial responses to nanoparticles, Gram-negative *E. coli* and Gram-positive *B. subtilis* treated with fourteen sizes and five concentrations of TiO<sub>2</sub> nanoparticles were studied. The end points of the microbial responses to photocatalytic nano-TiO<sub>2</sub> were investigated in terms of cell counting, lipid peroxidation of the cell membrane, cellular respiration, and enzymatic response, and DNA



damage. The results indicated that nano-TiO<sub>2</sub> particles in the absence and the presence of photoactivation induced cell inactivation, lipid peroxidation, cellular respiration disruption, increased enzymatic response, and oxidative DNA damages. In total darkness, lower levels of lipid peroxidation and DNA damage were detected than those in light condition. Results also showed that as irradiation time increased the photocatalytic effect on DNA damage and lipid peroxidation also increased. The lipid peroxidation and the simultaneous losses of both membrane-dependent respiratory activity showed that cell viability depend strictly on the presence of both light and TiO<sub>2</sub>. It can be concluded that TiO<sub>2</sub> photocatalysis promoted peroxidation of the polyunsaturated phospholipid component of the lipid membrane initially and induced major disorder in the bacteria cell membrane. Subsequently, essential functions that rely on intact cell membrane architecture, such as respiratory activity, were lost, and cell death was inevitable. The results also highlight the need for caution during the use and disposal of such manufactured nanomaterials to prevent unintended environmental impacts, as well as the importance of further research on the mechanisms and factors that increase ecotoxicity to enhance risk management.

**WP159 Impact of Organic Carbon on the Toxicity of Freshly Prepared and Stored Silver Nanoparticles** A.J. Kennedy, US Army Engineer Research and Development Center; J.K. Stanley, US Army Engineer Research and Development Center, Environmental Laboratory; A.C. Ryan, HDR|HydroQual; B.D. Meeks, Badger Technical Services; J.G. Laird, US Army Engineer Research and Development Center; A.J. Bednar, US Army Engineer Research and Development Center; A. Poda, US Army Engineer Research & Development Center, Waterways Experiment Station. In a previous investigation, we determined that the concentration of total measureable silver for various nanosilver suspensions was not predictive of toxicity. However, the fraction of the concentration measured following ultracentrifugation and by integration under field flow fractogram void peaks was comparable to dissolved silver (Ag<sup>+</sup>) toxicity. In the current study, we exposed *Ceriodaphnia dubia* to both 20 nm (hydrodynamic diameter of 27 nm) NanoComposix (San Diego, CA) citrate capped nanosilver and silver nitrate at multiple dissolved organic carbon (DOC) concentrations. A concentrated nanosilver stock was diluted to 10 mg/L using ultrapure water and was not amended with additional citrate. Using this freshly prepared stock, *C. dubia* were exposed to dissolved Ag<sup>+</sup> and nanoparticles at either 0 or 3.9 mg DOC/L; results suggested that both Ag<sup>+</sup> toxicity (significant increase in LC50 from 0.3 mg/L [0.2 – 0.3 ug/L] to 1.5 [1.3 – 1.8 ug/L] and nanosilver toxicity (LC50 increased from 16.9 [13.1 – 22.0 ug/L] to 23.7 [18.0 – 31.3 ug/L]) were reduced with higher DOC. However, when the same stored stock was reused one month later in a similar experiment, the toxicity of the nanosilver was significantly higher relative to the previous experiment, both at a lower DOC concentration of 2.0 mg/L (LC50 = 14.1 [11.1 – 18.8 ug/L]) and at 0 mg/L (LC50 = 5.1 [3.8 – 6.7 ug/L]). Dissolved Ag<sup>+</sup> toxicity was comparable between the two experiments. Additional experiments were conducted to test the hypothesis that nanosilver became more toxic due to dissolution caused by the dilution and storage of the stock without stabilizing the particles with additional citrate. This result, if repeatable, may have implications on the consistency of toxicity studies if stock handling and storage are not standardized. These data are being generated to consider the utility of the biotic ligand model to predict nanosilver toxicity.

**WP160 The Impact of Silver Nanowire Physicochemical Properties in Environmental Media and on Nanowire Toxicity to *Daphnia magna* and Fathead Minnow Cells** L.D. Scanlan, Univ of California, Berkeley, Molecular Toxicology, U.C. Berkeley, Molecular Toxicology, U.C. Berkeley, graduate student; A. Tagmount, U.C. Berkeley, Molecular Toxicology; B. Gilbert, Lawrence Berkeley National Laboratory; C. Clark, U.C. Berkeley, Molecular Toxicology; C. Vulpe, U.C. Berkeley. Nanowires are high-aspect-ratio inorganic nanomaterials that are distinct from nanoparticles (such as semiconductor quantum dots) and carbon-based materials (such as nanotubes). Nanowires are anticipated to have diverse technological applications, leading to increasing risk of environmental release during manufacture or from end products. Environmental contaminants pose particular risks to freshwater aquatic ecosystems and while there are now many studies of the environmental toxicity of nanoparticles there is very limited information on the potential toxicity of nanowires on aquatic organisms. We have performed an assessment of the environmental toxicity of silver nanowires (AgNW) by combining preliminary acute toxicity studies in the fresh water

ecoindicator *Daphnia magna* with studies of AgNW reactivity and aggregation in *Daphnia* media. We also used genomic tools to analyze differential gene expression in daphnids exposed to nanowires. The toxicity of AgNWs was additionally investigated at the cellular level in fathead minnow, another aquatic eco-indicator organism. We employed cell-based assays to assess and investigate the modes of action of AgNW cytotoxicity. Classical viability assays (e.g., MTT, MTS) as well as multiplexed cell-based imaging assays (High Content Screening) were employed to tackle this question. We are currently investigating in *D. magna* and fish cells whether or not AgNW functionalization and the ratio length to diameter influence the AgNW toxicity. A cell uptake study was also carried out to correlate the cytotoxicity with AgNW internalization.

**WP161 The Influence of Dissolved Organic Matter and Coating on the Toxicity of Silver Nanoparticles in *Daphnia magna*** K.M. Newton, Clemson Univ Institute of Environmental Toxicology (CU-ENTOX), Biological Sciences, Clemson Univ, Clemson Univ Institute of Environmental Toxicology; L. Sweet, Clemson Univ Institute of Environmental Toxicology (CU-ENTOX), Biological Sciences; A. Bone, B. Coleman, C. Matson, Center of Environmental Implications of Nano technology (CEINT); S. Klaine, Clemson Univ Institute of Environmental Toxicology (CU-ENTOX), Biological Sciences. Silver Nanoparticles (AgNPs) are by far one of the most widely used nanomaterials in general consumer products. Their inherent antimicrobial property has greatly increased their use in health and fitness products, household cleaning products, cosmetics and personal care products. The consequence of increased use is that of increased inputs into aquatic ecosystems. To date, a lot of uncertainty still clouds the ecological impact of AgNPs. However, emerging research on their fate and behavior in aqueous systems points to the release of silver ions (Ag<sup>+</sup>) during use and on disposal of products. Different sources of dissolved organic matter (DOM) and nanomaterial coating may affect the bioavailability of Ag<sup>+</sup> in aqueous systems and thus its toxicity. In order to investigate the roles that different sources of dissolved organic matter and AgNP-coating play in silver toxicity, microcosms were prepared using four different scenarios: water only, sediment + water, plants + water, and plants+sediment+water. Treatments were prepared in triplicate: Untreated controls and 2.5 mg/L Ag as AgNO<sub>3</sub> (Ag-I), Gum Arabic coated (Ag-GA) and Polyvinylpyrrolidone coated (Ag-PVP) nanoparticles for each of the scenarios. 48-hour acute toxicity tests in *D. magna* indicated AgNO<sub>3</sub> was the most toxic, that Ag-PVP was less toxic than Ag-GA in moderately hard water (MHW) and in both cases their toxicity was reduced in the presence of DOM. The presence of plant organic matter in general reduced the toxicity of AgNPs however, the reduction was not quantitatively related to DOC.

**WP162 Assessment of Silver Ion Toxicity Using a Novel Method to Fish Embryos** Y. Nakagame, T. Ariyoshi, T. Niwa, S. Nagasaka, H. Takei, S. Kashiwada, Toyo Univ, Dept of Life Sciences. The use of nanotechnology is increasing in both high-tech industries and medicine. While such technology offers many benefits, there are concerns regarding the potential environmental and human health risks associated with exposure to nanomaterials. Silver nanomaterials including nanocolloids and nanoparticles are ones of the most widely used materials, due to their disinfectant properties, and are likely to enter and impact the aquatic environment through municipal and other wastewater streams. Although silver is rare in the natural environment, concentrations have been elevated due to anthropogenic activities. Despite the widespread use of silver and silver ions in industry and for medical purposes, only limited information on silver toxicity is available. Existing environmental and human studies seem to demonstrate that free silver ions (Ag<sup>+</sup>) are more toxic than others. However, it is not easy to test toxicity of Ag<sup>+</sup> only because of existence of base, a counterpart of acid, which lead to acidification of test water and cause toxic effects as well. In use of AgNO<sub>3</sub> for source of Ag<sup>+</sup>, it is impossible to eliminate biological effects of NO<sub>3</sub><sup>-</sup> from toxicity data. Herein, we will demonstrate a newly developed method for testing of Ag<sup>+</sup> toxicity. Using a nanotechnology, Ag nanoparticles are formed on bottoms of a 6 well-plate. Using this "bottom-fixed Ag nanoparticles plate" released Ag<sup>+</sup> (which should be pH-dependent) into ambient water from the bottom were tested and toxic effects on medaka fish embryos were estimated away from Ag nanoparticles. In case of ERM (embryo rearing medium) as ambient water, less-development of eyes, short spinal cord, vascular defects, blood clots, percardiovascular edema, ischemia, short body lengths, reduction of heart rates, and hatching delay were observed significantly more in pH4 than pH9. These features were similar to those of

Ag nanocolloids exposure. Interestingly, in case of ultrapure water instead of ERM, tested embryos exhibited complete mortality within 12 hrs in pH4, 7 and 9. If medaka embryos were incubated in ultrapure water, embryos could survive only for 2-3 days in pH4, and 13.3% and 11.1% of mortalities were shown in pH7 and pH9, respectively. This would be because ERM (Na<sup>+</sup> and/or Ca<sup>++</sup>) could mitigate Ag<sup>+</sup> toxicity in comparison to ultrapure water. The new developed method would be useful for elucidation of Ag toxic mechanism and assessment of Ag<sup>+</sup> toxicity to aquatic organisms.

**WP163 Multiple Fish Species and Life Stage Assessment of Nanosilver Toxicity** A.D. Hawkins, Univ of Mississippi, Dept of Pharmacology, Environmental Toxicology Research Program; J.D. Laird, US Army Engineer Research & Development Center, SpecPro Incorporated, US Army Engineer Research & Development Center, Environmental Laboratory; C. Thornton, Univ of Mississippi, Dept of Pharmacology and Environmental Toxicology Research Program, Univ of Mississippi, Pharmacology; J.A. Steevens, US Army Engineer Research & Development Center, Waterways Experiment Station; K.L. Willett, Univ of Mississippi, Environmental Toxicology Research Program, Univ of Mississippi, Dept of Environmental Toxicol. Recent advancements in nanotechnology have lead to an increase of "nanoe-enabled" goods on the market. Silver nanoparticles are the most widely used nanoparticles because of their antibacterial and antifungal properties. Silver is known for acute and long-term toxicity, however, little is currently known about potential developmental or gill toxicity of nanosilver exposure. Here, we investigated two species and two lifestages for effects of nanosilver relative to silver nitrate. First, adult fathead minnows (*Pimephales promelas*) were exposed for 96 hr with daily water renewals to polyvinylpyrrolidone coated silver nanoparticles (PVP-AgNPs) or silver nitrate (AgNO<sub>3</sub>) at 3 different nominal concentrations (6, 80, and 214 µg/L) or (1.5, 4, and 6 µg/L), respectively. The gills were processed for histopathological detection of lesions including presence of hyperplasia, hypertrophy, circulatory disturbances, and fusion of lamellae and for enzymatic measures of oxidative stress. The CytoViva hyperspectral imaging system and ICP-MS are also being used to quantify the presence of nanosilver in the gills and assess the difference in uptake from the different silver solutions. Second, Japanese medaka (*Oryzias latipes*) embryos were exposed to 3 nominal concentrations (1, 10 and 100 µg/L) of PVP-AgNPs, AgNO<sub>3</sub>, and Silver Biotics (ASAP) for 7 days. These embryos were scored at 7 days postfertilization for lethality and cardiac toxicities (blood clot, edema and tube heart using a deformity index). Associated gene expression changes of metallothionein (MT), glutathione S transferase (GST), heat shock protein 70 (HSP70), and transferrin (TF) are being measured by qRT-PCR of whole embryos. Lastly, we will consider the physiological consequences of changing pH, Ca<sup>2+</sup>, Cl<sup>-</sup>, DOC and sulfide during exposure to nanosilver. Together this work will identify both potential species and developmental stage sensitivities to nanosilver exposure and begin to identify possible mechanisms of toxicity. Supported by US Army ERDC #W912HZ-09-C-0033

**WP165 Comparing Mechanisms of Toxicity and Effects of Silver Nanoparticles and Ionic Silver on *Daphnia magna* Using Non-invasive Real-time Methods** M.C. Stensberg, Purdue Univ, Dept of Agricultural and Biological Engineering; E.S. McLamore, Univ of Florida – Gainesville, Agricultural and Biological Engineering Dept; Q.C. Wei, A.C. Wei, Purdue Univ, Dept of Chemistry; D.M. Porterfield, Purdue Univ, Dept of Agricultural and Biological Engineering; M.S. Sepulveda, Purdue Univ, Dept of Forestry and Natural Resources. Silver nanoparticles (AgNPs) are becoming increasingly popular as antimicrobial agents in hundreds of products; however, the mechanisms of toxicity and potential health effects of AgNPs to other organisms are not fully understood. Many traditional toxicological studies are invasive and destructive. The goal of this research was to compare the mechanisms of toxicity and effects induced by ionic silver (as silver nitrate, AgNO<sub>3</sub>) and AgNPs in the water flea, *Daphnia magna* using non-invasive real-time sensing methods. *D. magna* embryos (stages 1-2) were exposed to sublethal concentrations (130 ng/L and 650 ng/L) of AgNO<sub>3</sub> and AgNPs. Particle uptake was monitored using confocal reflectance microscopy; growth with differential imaging; and effects on physiological fluxes of oxygen and hydrogen quantified using self-referencing microsensors. AgNPs (650 ng/L) were observed inside the embryos as early as 3 hours post exposure. AgNO<sub>3</sub> (130 ng/L) significantly impaired embryo growth over the course of 25 hours. Although no changes were observed in oxygen flux after exposure to either AgNPs or AgNO<sub>3</sub>, proton flux increased in both treatments within minutes after the initial exposure, but significantly

more so in embryos exposed to AgNPs. Taken together these results indicate that AgNPs move into the embryos within minutes after exposure and elicit responses in proton flux that are not translated in growth effects suggesting differences in the mechanism of toxicity between AgNO<sub>3</sub> and AgNPs.

**WP166 Detection of Naturally Internalized Nanoparticles in Aquatic Organisms** A. Harmon, US Army Engineer Research and Development Center, Environmental Laboratory; A.J. Kennedy, US Army Engineer Research and Development Center; J.D. Laird, US Army Engineer Research & Development Center, SpecPro Incorporated, US Army Engineer Research & Development Center, Environmental Laboratory; J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center, Research Biologist; J.K. Stanley, US Army Engineer Research and Development Center, Environmental Laboratory; M. Hull, NanoSafe, Inc; A.J. Bednar, US Army Engineer Research and Development Center; B.D. Meeks, Badger Technical Services; J.A. Steevens, US Army Engineer Research & Development Center, Waterways Experiment Station. Numerous studies have investigated the toxicity and bioaccumulation potential of engineered nanoparticles. It is important to develop techniques to evaluate what form of the originally dosed nanoparticle may exist within the tissues of organisms (e.g., primary particle, aggregate, bulk, ion) to determine appropriate accumulation factors and populate environmental risk models. This investigation focuses on two different techniques that were employed to characterize the state of nanoparticles in organism tissue: hyperspectral microscopy (CytoViva<sup>®</sup>, Auburn, AL) of tissue sections of nanoparticle exposed animals and field-flow-fractionation coupled with ICP-MS of deionized water extracted tissue. Organisms were exposed to nanoparticles (TiO<sub>2</sub>, nanosilver) using a peristaltic pump to maintain as consistent as possible particle concentrations and suspension characteristics. Smaller and larger particle aggregate states were created by dispersing nanoparticles in low (0 – 100 µS/cm) and higher ionic strength (280 – 560 µS/cm) water. The organisms exposed included zebrafish (*Danio rerio*), fathead minnows (*Pimephales promelas*), zooplankton (*Daphnia magna*) and the Asiatic clam (*Corbicula fluminea*). Polyvinylpyrrolidone (PVP) nanosilver and TiO<sub>2</sub> particles were detected by hyperspectral analysis based on their unique spectral signatures on the gill filaments of *P. promelas* and *D. rerio*, respectively. Similarly, TiO<sub>2</sub> particles were also detected using hyperspectral imaging in cross sections of *D. rerio* and *C. fluminea* gastrointestinal tracts. The more dispersed TiO<sub>2</sub> particles remained in smaller aggregates that became entrapped within *D. rerio* intestinal folds or villi, with some particles potentially absorbed into the lining. The more aggregated TiO<sub>2</sub> particles continued to form larger aggregates that completely filled the intestines, potentially leading to an impacted gastrointestinal tract. Future studies will investigate additional particle and aggregate sizes to evaluate potential differences in uptake and assimilation efficiency of nanoparticles. Such knowledge should lead to more informed estimates of bioaccumulation and trophic transfer factors to populate databases such as NanoExPERT.

**WP167 Investigating the Fate and Toxicological Effects of Nano-TiO<sub>2</sub> in *Daphnia magna*** T. Blickey, National Institute of Standards & Technology (NIST), Surface and Microanalysis Science; C. Szakal, R. Holbrook, National Institute of Standards & Technology (NIST) The literature suggests that nano-sized titanium dioxide is acutely toxic to *Daphnia magna* at concentrations greater than 100 mg/L, which is well above what is considered environmentally-relevant (0.7-16 µg/L). We are chronically exposing this freshwater invertebrate to low levels of TiO<sub>2</sub> (1-1000 µg/L) to determine sub-lethal toxicological effects. Nano-sized TiO<sub>2</sub> (NIST SRM-1898) suspensions were prepared by sonication, coated in bovine serum albumin (BSA), and dispersed in PBS, thus yielding particles that were ~80 nm in diameter. To determine particle behavior in the water column, the material was mixed into OECD *Daphnia* media, transferred to graduated cylinders, and measured for concentration via UV/VIS spectroscopy at 1, 24, 48, and 168 hours. Negligible settling occurred during the first 48 hours; however, 65% of the total suspended material had precipitated to the bottom layer by day 7. Ongoing studies involve aqueously exposing *Daphnia* neonates to these BSA-TiO<sub>2</sub> suspensions for 14 or 21 days. Endpoints include molting, time to first brood, and reproduction. At the conclusion of the exposures, daphnids are rinsed with clean OECD media before excising their eggs. The eggs are currently being evaluated for TiO<sub>2</sub> deposition with Focused Ion Beam/Secondary Ion Mass Spectrometry (FIB/SIMS). Additionally, adult *Daphnia* are being aqueously exposed to 1 mg/L BSA-TiO<sub>2</sub> for 7 days and the gut tract contents are examined for transformation of the particles with

**SIMS.** The goal of these studies is to determine where the nanoparticles reside in the aquatic environment as well as where and in what form the particles exist within the organism.

**WP168 Photo Enhanced Toxicity of Nano Titanium Dioxide (Anatase) on Freshwater Zooplankton** M. Alloy, Univ of North Texas, Dept of Biological Sciences; A. Roberts, Univ of North Texas. Classic photo-enhanced toxicity is focused around PAHs closely associated with petroleum use, internal combustion engines, oil refining, and oil spills. The majority of nanotoxicology has been focused on effects of nanomaterials in acute exposures. Titanium dioxide has been used for decades as a paint pigment, food coloring, sun screen, the active portion of self-cleaning surfaces, fog free mirrors, and in many more roles prior to the use of nano-scale TiO<sub>2</sub> crystals. The first reports of TiO<sub>2</sub> photocatalytically decomposing compounds in the aqueous phase by generating reactive oxygen species was reported in the late 1970s. Toxicology of nano TiO<sub>2</sub> is not new, but few studies have looked at aquatic toxicity of nano TiO<sub>2</sub> under exposure to UV light. The goals of this study are: to determine if nano TiO<sub>2</sub> is more toxic to aquatic organisms in the presence of UV light, if it follows classic phototoxicant patterns of reciprocity, and to what magnitude does UV exposure mediate toxicity. Time to failure tests using *Daphnia magna* neonates as model organisms were run and LC<sub>50</sub> values established under a variety of exposure conditions. Results of these tests indicate that TiO<sub>2</sub> is phototoxic to zooplankton in aquatic environments at relatively low concentrations.

**WP169 Influence of Functionalization on the Suspension Stability of Titanium Dioxide Nanoparticles in Aqueous Matrices** S.P. Yang, Univ of Wisconsin, Molecular and Environmental Toxicology; K.M. Louis, Univ of Wisconsin; O. Bar-Ilan, Univ of Wisconsin, Division of Pharmaceutical Sciences; R.J. Hamers, Univ of Wisconsin, Chemistry; R.E. Peterson, W. Heideman, Univ of Wisconsin, School of Pharmacy; J.A. Pedersen, Univ of Wisconsin, Soil Science. The ability of titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) to adsorb ultraviolet light and generate reactive oxygen species has led to applications ranging from cosmetics to wastewater treatment to photovoltaics. As such, production volumes are expected to surpass 10,000 tons per year by 2014. Many applications require stable NP suspensions, and the addition of organic ligands during synthesis can increase stability through electrostatic and steric repulsion. Upon release into aquatic environments, NP stability can be modified by solution conditions (e.g., pH, ionic strength, dissolved organic matter (DOM)). These factors are expected to impact NP environmental fate and bioavailability. Here, we examine the influence of functionalization on TiO<sub>2</sub> NP suspension stability as a function of ionic strength and composition, DOM concentration, and sunlight exposure. We synthesized TiO<sub>2</sub> NPs from TiCl<sub>4</sub> and were functionalized with various organic ligands (e.g., citrate, dihydroxybenzaldehyde (DHBA), polyethylene glycol). Dynamic and electrophoretic light scattering were employed to assess suspension stability and a metal halide lamp designed to mimic sunlight at an underwater depth of 1-2 m. Suspensions of DHBA- and citrate-functionalized TiO<sub>2</sub> NPs (primary particle size: 5-6 nm) were stable in NaCl concentrations up to 1 M. However, suspensions were destabilized by low concentrations (0.1 mM) of Ca<sup>2+</sup>. For the DHBA and citrate functionalizations, dissolved organic matter (Suwannee River natural organic matter isolate; 10 mg L<sup>-1</sup>) increased suspension stability in the presence of Ca<sup>2+</sup> ions (0.1 mM). In the absence of DOM, exposure to simulated sunlight increased the aggregate size of DHBA- and citrate-functionalized NPs. These results suggest the TiO<sub>2</sub> NPs can photocatalytically degrade the functionalizations. Changes in TiO<sub>2</sub> NP aggregate size through these various mechanisms are expected to impact uptake by aquatic organisms.

**WP170 Effect of ZnO and TiO<sub>2</sub> Nanoparticles on Larva and Embryo of *Oryzias latipes* Under Continuous Ultraviolet Irradiation** Y. Shin, Konkuk Univ; Y. An, Konkuk Univ, Dept of Environmental Science. We investigated the effect of zinc oxide and titanium oxide nanoparticles on the early development of *Oryzias latipes* when the fish embryo rearing media were continuously illuminated by ultraviolet irradiation during exposure period. UV irradiation was conducted in a controlled photoreactor where the intensity of UV was low enough not to influence the test species. Control experiment was conducted in visible light condition. We observed that abnormal development of bladder was increased in nanoparticle solutions. No significant decrease in hatchment was found under the UV irradiation. This study indicates that zinc oxide and titanium oxide nanoparticles can influence on the larval development of *Oryzias latipes* in aquatic ecosystem

when they are exposed to sunlight having UV region. This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2009-0079204).

**WP171 Effect of ZnO and TiO<sub>2</sub> Nanoparticles to the Green Microalgae *Pseudokirchneriella subcapitata* Under Visible Light, UVA, and UVB Irradiations** W. Lee, Konkuk Univ, Konkuk Univ, Dept of Environmental Science; Y. An, Konkuk Univ, Dept of Environmental Science. Metal oxide nanoparticles have been widely used in a variety of industries. ZnO and TiO<sub>2</sub> nanoparticles are photoreactive substances that are of raising concerns regarding phototoxicity. We assessed the ecotoxicity of ZnO and TiO<sub>2</sub> nanoparticles to green microalgae *Pseudokirchneriella subcapitata* under different irradiation conditions. Nanoparticles were dispersed in OECD algae medium, and the test units were preilluminated by UVA and UVB light in a photoreactor. Visible light irradiation was served as a control. Inhibition of algae growth was related with the ZnO and TiO<sub>2</sub> NPs concentrations. The growth of *P. subcapitata* was found to be inhibited under visible light, UVA, and UVB irradiation conditions, with no significant differences in results among the light conditions. This reason was attributed to the photocatalytic activity of ZnO and TiO<sub>2</sub> NPs in both UV and visible ranges. This study provides us with a better understanding of toxicity of photoreactive nanoparticles as related to the effects of light. This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2009-0079204)

**WP172 Assessing Genotoxicity of Nanosized ZnO and TiO<sub>2</sub> in the SOS Chromotests** S. Nam, Konkuk Univ; Y. An, Konkuk Univ, Dept of Environmental Science. Zinc oxide nanoparticles (ZnO NPs) and titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) are widely used in cosmetic products such as colorants and sunscreens. These NPs can be absorbed to users' bodies through oral, inhalation, or dermal contact pathways. The presence of genotoxics entering into the body can induce a probable problem since they cause DNA damage of the receptors. The SOS chromotest with *Escherichia coli* PQ37 has been used for the screening of a variety of chemicals because of its practicality. However, available SOS chromotest data for nanomaterials is extremely limited. In this study, we investigated the genotoxicity of ZnO NPs and TiO<sub>2</sub> NPs by the SOS chromotest with the *E. coli* PQ37 strain. The exposure concentrations of chemicals were 0-100 mg/L ZnO NPs and TiO<sub>2</sub> NPs. Additionally, zinc ion were assessed in the same ways of ZnO NPs. The genotoxicity of Ti ion was not assessed because of extremely low solubility of TiO<sub>2</sub> NPs. ZnO, TiO<sub>2</sub>, and Zn ion in a range of tested concentrations (between 0 and 100 mg/L) exerted no effects on the SOS response in the SOS chromotest, evidencing IFmax values of 0.90, 1.24, and 0.68, respectively. Therefore, nanoparticles of ZnO and TiO<sub>2</sub> and zinc ion are classified as non-genotoxics. This is, to the best of our knowledge, the first report to evaluate the genotoxicity of ZnO NPs and TiO<sub>2</sub> NPs using a SOS chromotest. This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2009-0079204)

**WP173 Toxicity of ZnO Nanoparticles to Estuarine Amphipod (*Leptocheirus plumulosus*)** S.K. Sundaray, Chonnam National Univ, Dept of Oceanography; B. Lee, Chonnam National Univ, Oceanography; S. Yang, S. Lee, N. Kim, Chonnam National Univ, Dept of Oceanography. Zinc oxide nanoparticles (ZnO-NPs) are now widely used in many industrial, household, and cosmetic products, and there are increasing concerns of their environmental fate and potential toxicities in aquatic organisms. The objective of the present study was to evaluate the potential toxicological effects of ZnO-NPs (< 30 nm) compared to ionic zinc (Zn<sup>2+</sup>, ZnCl<sub>2</sub>) in the estuarine amphipods (*Leptocheirus plumulosus*). In the present study, 10-d amphipod sediment acute toxicity test was conducted referred to the USEPA guidelines, using estuarine sediments spiked with 5 nominal Zn concentrations of 5, 10, 15, 30 and 60 mmol/g for both types of Zn. No significant mortality was observed in this 10 d acute toxicity test, when the amphipods were exposed up to 5 µmol Zn/g as ZnO-NPs. Toxicity experiments revealed comparable toxicity for ionic Zn<sup>2+</sup> (in terms of pore water, PW) spiked as ZnCl<sub>2</sub> (5-15 µmol/g), with a 96-h median lethal concentration (LC50) value (60 mM), attributable solely to dissolved zinc. 100% mortality was observed in 60 µmol Zn/g for spiked ZnO-NPs with 40-49 mM Zn in PW, while that for spiked ZnCl<sub>2</sub> it was 15 µmol Zn/g as ZnCl<sub>2</sub> with 166-434 mM Zn in PW. Based on total Zn concentration, toxicity was 3-4 times higher for ZnCl<sub>2</sub> than for ZnO-NPs. However, when compared as a function of the Zn<sup>2+</sup> concentration in PW, toxicity of ZnO-NPs appeared to be



much higher than that of  $\text{ZnCl}_2$ . These results suggest that  $\text{ZnO-NPs}$  play a significant and critical role in acute toxicity. During the 10 d experiments, Zn concentration was increased (up to two fold) in overlying water (OW) for spiked  $\text{ZnO-NPs}$ , whereas that for spiked  $\text{ZnCl}_2$  remained relatively constant. This may be caused by the release of  $\text{Zn}^{2+}$  from spiked  $\text{ZnO-NPs}$  sediments into the OW. Concentrations of Zn in PW decreased significantly in case of spiked  $\text{ZnCl}_2$  (by 2.3 to 3.2- fold) as compared to spiked  $\text{ZnO-NPs}$  (by 1.2 to 2.1- fold). The apparent partitioning coefficient ( $K_{pw}$ , ratio of [Zn] in SEM to PW) increased with increased  $\text{Zn}^{2+}$  in spiked  $\text{ZnCl}_2$  sediments and decreased with time, whereas that for spiked  $\text{ZnO-NPs}$  remained relatively constant. These results reveal that dissolution of  $\text{ZnO-NPs}$  from spiked sediments relatively increases with time and leads to potential toxicity

**WP174 Biodistribution of Quantum Dots in Fathead Minnows, *Pimephales promelas*, Using a Fluorescence Based Assay** C.M. Lavelle, Univ of Florida, Center for Environmental Health and Toxicology; M. Hahn, Univ of Florida, Particle Engineering Research Center; K.J. Kroll, Univ of Florida, Physiological Sciences; K. Powers, Univ of Florida, Particle Engineering Research Center; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. The abundant use of nanomaterials in a variety of applications including cosmetics, electronics, and biomedicine necessitates the understanding for their interactions in biological systems. Of specific importance are the biological transport and fate of nanomaterials in aquatic organisms, as the aquatic environment is a common sink for environmental contaminants. Furthermore, aquatic vertebrates may serve as a model species for the effects of nanomaterials in other organisms. Previous research has shown that particle characteristics such as size and surface chemistry influence biological fate and transport and ultimately effects. One class of nanomaterials of specific interest are the semiconductor quantum dots (QD) which consist of a semiconductor core and shell that can be modified by the addition of different functional groups ultimately changing their surface chemistry. Here we investigated the transport and fate of QDs by utilizing the inherent fluorescent qualities of commercially available quantum dots (QD 705, Invitrogen) with the development of a fluorescence based assay. Biotransportation of QDs was elucidated by dosing of QDs with different surface properties by oral gavage into fathead minnows, *Pimephales promelas*. At multiple time points post dosing, tissues were harvested and homogenized by sonication and later digested with 5N NaOH. Digested tissues were analyzed for QDs by measuring fluorescence (filters: 355excitation, 685 emission). Standard curves were constructed using spiked control tissue pools for each tissue type examined. Results show that most of the dosed QDs are passed through the gut and detectable in the feces at 12 hours post gavage, however, some are able to cross the gut membrane resulting in higher fluorescence readings in the blood as well as in various other tissues in a manner dependent upon QD type and fish sex. Comparison of this fluorescence assay with another widely used technique using the Xenogen platform highlights the increased sensitivity of the fluorescence assay. Results of the fluorescence assay can be further validated with the use of other techniques including confocal microscopy, TEM, and ICP-MS.

**WP175 Interactions of Free Chlorine with  $\text{nC}_{60}$  Differs Between Aged Versus Fresh Aqueous  $\text{nC}_{60}$  Assessed by Differences in Chlorine Toxicity in Zebrafish** J. Park, Univ of Tennessee, Center for Environmental Biotechnology; M. Barham, F. Menn, The Univ of Tennessee, Center for Environmental Biotechnology; Q. He, Univ of Tennessee, Center for Environmental Biotechnology; T. Henry, Univ of Plymouth, School of Biomedical and Biological Sciences, Univ of Plymouth, School of Biology, Univ of Plymouth, School of Biological Sciences; G.S. Saylor, Univ of Tennessee, Center for Environmental Biotechnology, Dept of Microbiology, Dept of Ecology and Evolutionary Biology. A substantial release pathway for manufactured nanoparticles (NPs) is via wastewaters, and passage through wastewater treatment plants (WTPs) will occur before NPs enter surface waters. The  $\text{C}_{60}$  fullerene is a NP that is present in the aqueous phase as aggregates of many  $\text{C}_{60}$  molecules ( $\text{nC}_{60}$ ), and  $\text{nC}_{60}$  are expected to arrive and pass through WTPs; however, little is known about the effects of treatment processes on  $\text{nC}_{60}$  aggregate characteristics or the fate of substances (e.g., co-contaminants) that are associated with  $\text{nC}_{60}$  as they pass through WTPs. One treatment process of WTPs is chlorination, and the main objective of the present study was to investigate the interaction of aqueous  $\text{nC}_{60}$  with free chlorine and evaluate the toxicity in larval zebrafish. We prepared 6 treatments; 1) control (no chemical addition), 2) positive control (5 mg/L of

free chlorine), 3) aged  $\text{nC}_{60}$  (nominal 3 mg/L  $\text{nC}_{60}$  suspended in water for 3 years), 4) fresh  $\text{nC}_{60}$  (nominal 3 mg/L  $\text{nC}_{60}$  solution suspended in water for 2 days), 5) aged  $\text{nC}_{60}$ +free chlorine, and 6) fresh  $\text{nC}_{60}$ + free chlorine. Aqueous  $\text{nC}_{60}$  and free chlorine were mixed overnight prior to exposure. Mortality and hatching rates were monitored over 5 d in zebrafish eggs (1-day post fertilization) exposed to the above solutions. The presence of aged or fresh  $\text{nC}_{60}$  did not affect hatching rate. As expected, free chlorine was acutely toxic in zebrafish with dose- and time-dependence (100% mortality within 1 h at 0.24 mg/L free chlorine). However, the acute toxicity of chlorine was significantly reduced with the co-exposure of aged  $\text{nC}_{60}$ , but not with that of fresh  $\text{nC}_{60}$ , suggesting different associations between  $\text{nC}_{60}$  and free chlorine for aged and fresh aqueous  $\text{nC}_{60}$  preparations. To investigate the physicochemical factors underlying the differences in toxicity, ongoing efforts are studying aged and fresh  $\text{nC}_{60}$  with Fourier Transformation Infrared Spectroscopy (FT-IR), Nuclear Magnetic Resonance (NMR), and Scanning Electron Microscope (SEM). The reduction in the acute toxicity of free chlorine when mixed with aged  $\text{nC}_{60}$  but not fresh  $\text{nC}_{60}$  is further indication that that physicochemistry of aqueous  $\text{nC}_{60}$  changes with time.

**WP176 Impacts of Chronic Exposures to  $\text{C}_{60}$  and  $\text{C}_{60}$ -OH Nanoparticles on *Daphnia magna*: Relating Gene Expression and Mortality for Ecological Risk Assessments** B.J. Blalock, Univ of Massachusetts, Boston, Environmental, Earth, and Ocean Sciences; D. Arndt, R. Klaper, Univ of Wisconsin-Milwaukee, Great Lakes WATER Institute, School of Freshwater Sciences. Fullerene ( $\text{C}_{60}$ ) and hydroxylated fullerene ( $\text{C}_{60}$ -OH) nanoparticles have a wide range of applications and a great potential for medical benefits. However, the increasing manufacturing and production of fullerenes has led to the need for more research to assess the associated environmental impacts and determine which nanoparticles are safer for industry, consumer goods, and medicine. Since chronic exposures are more representative of the field this study aims to relate mortality data and Glutathione-S-transferase (GST) and Catalase (CAT) gene expression levels to use genomic data as biomarkers for fullerene exposure. This experiment is a 19-day static renewal chronic toxicity assay using *D. magna* as the indicator species. *D. magna* are exposed to 1 mg/L, 10 mg/L, and 50 mg/L treatments of  $\text{C}_{60}$ -stir and  $\text{C}_{60}$ -OH nanoparticles. At the end of the exposure mRNA is extracted and reverse transcribed into the more stable cDNA and the gene expression levels are examined for the selected primers and normalized to Actin.  $\text{C}_{60}$ -stir and  $\text{C}_{60}$ -OH nanoparticles are characterized by TEM and Zetasizer. The results of this experiment indicate significant GST and CAT gene expression levels and percent mortality at 10 and 50 mg/L concentrations of  $\text{C}_{60}$ -stir. Therefore, GST and CAT gene expression levels can potentially be used as biomarkers of  $\text{C}_{60}$ -stir exposure. Since it is difficult to quantify the concentration of nanoparticles in aquatic systems, GST and CAT gene expression levels can potentially be used as a threshold to rank and characterize nanoparticle risk in aquatic environments.

**WP177 The Occurrence of Lead in Soil and Vegetables at a Community Garden in Omaha, Nebraska** J. Sangster, Univ of Nebraska – Lincoln, Dept of Civil Engineering; S. Bartelt-Hunt, Univ of Nebraska, Civil Engineering; A. Neilson, Univ of Nebraska – Lincoln, Civil Engineering. Lead is a persistent and ubiquitous soil pollutant in urban environments and is of significant public health importance as it acts as a neurotoxin, particularly to children. Elevated lead concentrations in soil may be due to the use of lead-based paint, emissions from combustion of leaded gasoline, industrial emissions, waste incineration, and pesticide application. One potential pathway for exposure can be consumption of produce grown in lead-contaminated soil, although there is relatively little information available about what types of produce accumulate more lead relative to others and the relationship between soil lead concentrations and concentration of lead in produce. The purpose of this project was to utilize undergraduate students in the environmental engineering laboratory course at the Univ of Nebraska to sample soil and vegetables obtained from City Sprouts, a community garden, located in Omaha, Nebraska. The garden is located within the boundaries of the Omaha Lead Superfund site, which consists of a 27 square mile area in Eastern Omaha with elevated lead concentrations in soil. This area encompasses 15,000 residential properties and was placed on the National Priorities List in 2003. Students collected, dried, and extracted total lead from soil and vegetable samples using a nitric acid/ hydrogen peroxide extraction procedure. The liquid extracts were analyzed for total lead using atomic absorption spectroscopy (AAS). The concentrations of lead measured in soil ranged from 4 to 574 parts per million (ppm). With a few exceptions,

most of the soil samples had lead concentrations below the action level set by the United States Environmental Protection Agency (USEPA) for Omaha, NE at 400 ppm. There was some variability in lead concentrations in soil samples obtained from a single bed. Some of the variability may be due to wind-blown soil contamination from an off-site location. Based on the limited number of vegetable samples tested, it is difficult to draw conclusions based on this data. There is not currently any regulatory limit on lead in food, however, a limit of 0.5 ppm has been established by the FDA for lead in glazed dinnerware. Based on this limit, the samples of leafy greens, eggplant, okra, and one of the tomato samples would be over this limit. Additional testing of vegetables, including more replicate samples would be appropriate.

**WP178 Biochar Amendment for Reduction of Bioavailability of Toxic Chemicals in Biosolids** H. Hwang, Univ of California, Davis, Dept of Civil and Environmental Engineering, Univ of California, Dept of Civil and Environmental Engineering; H. Kim, Univ of California, Civil and Environmental Engineering. Large fraction of sludge from municipal wastewater treatment plant is being converted to biosolids to use as fertilizer. Most biosolids are being applied to farmland but a certain portion of biosolids is mixed to soils for household gardening and municipal landscape. Biosolids are highly enriched with variety of hydrophobic toxic chemicals, including PBDEs, pyrethroid pesticides, phthalates, perfluorinated compounds, pharmaceuticals, personal care products, and trace metals. To investigate desorption and bioavailability of toxic chemicals in biosolids and potential of biochar amendment for reduction of their bioavailability, grass growing tests were conducted. Biochar is known to have greater capacity to hold organic contaminants and trace metals so plant uptake rate is expected to be reduced when biochar is mixed with biosolids before soil amendment. Biosolid amendment significantly increased the growth rate of grass. Biochar amendment also significantly increased the growth rate of grass. When both biosolids and biochar are added together, grass growth rate was slightly higher compared to that in soil amended with biosolid only. Next step is to measure toxic chemicals, including PBDEs, PAHs, pesticides, pharmaceuticals and trace metals accumulated in grass tissue to evaluate whether biochar amendment can reduce bioavailability of toxic chemicals in biosolids. The chemical measurement data will be added for the presentation.

**WP179 PCBs, PBDEs, and PAHs in the Urban Atmosphere: Spatial and Seasonal Trends and Implications for Contaminant Transport** L. Melymuk, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; M. Robson, Univ of Toronto, Dept of Geography and program in planning; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch; M.L. Diamond, Univ of Toronto, Dept of Geography and program in planning, Univ of Toronto, Dept of Geography. We have examined the distribution of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs) in the atmosphere of Toronto, Canada and the surrounding suburban/rural area. A series of temporally- and spatially-distributed air samples were collected over a 1-year period with polyurethane foam passive air samplers and high-volume active air samplers. Bulk air concentrations of  $\Sigma$ PAHs ranged from 0.27 ng/m<sup>3</sup> up to 51 ng/m<sup>3</sup>. Concentrations of  $\Sigma$ PCBs ranged from 6.0 pg/m<sup>3</sup> to 1300 pg/m<sup>3</sup>, and concentrations of  $\Sigma$ PBDEs ranged from 0.47 pg/m<sup>3</sup> to 110 pg/m<sup>3</sup>. All compounds exhibited highest concentrations in the urban core, and lowest concentrations in the surrounding rural areas. Urban-rural gradients were modeled using a radial dilution model, and the comparison between measured and modeled gradients was used to estimate the distribution of POP sources across the urban area. The measured gradient of PCBs in air followed the radial dilution model well, indicating that the dominant PCB source region in Toronto is the downtown core. However, the radial dilution model significantly underestimated atmospheric concentrations of PAHs at distances from 10–40 km from the downtown core, indicating a significant influence of diffuse PAH sources located throughout the urban/suburban region. Comparison between measured and modelled distributions of PBDEs indicated that the majority of sources were in the downtown area, with a lower source density in the suburban regions, and emphasized the importance of particle movement in the transport of PBDEs from the urban to surrounding regions. Relationships between atmospheric concentrations and meteorological parameters were also examined. A strong inverse relationship existed between temperature and partial pressure of the gas-phase PCBs

and PBDEs, indicating the dominant influence of temperature-controlled processes on air concentrations, in particular volatilization from local sources of PCBs and PBDEs. The relationship between temperature and gas-phase PAHs varied along the urban-rural gradient, which we believe indicates that in highly urbanized areas, such as downtown Toronto, volatilization from paved surfaces has a significant impact on air concentrations, while in areas with less impervious surface coverage the emissions from domestic heating have a greater influence on atmospheric concentrations of PAHs.

**WP180 Reconnaissance Study of Sealcoat Application in Toronto, Canada and an Estimate of Related PAH Emissions** E. Goosey, Univ of Toronto; M.L. Diamond, Univ of Toronto, Dept of Geography and program in planning, Univ of Toronto, Dept of Geography; S.A. Csiszar, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; S. Verkoyeon, C. Catching, Univ of Toronto; P.A. Helm, Ontario Ministry of the Environment, Environmental Monitoring and Reporting Branch. Coal tar sealcoats (CTS) are a significant source (i.e., ~50%) of PAH to urban surface waters in many Eastern US cities. This study reports the concentrations of PAH identified in paved surface materials collected from Toronto, Canada. These data are compared to different PAH environmental sources and are used to examine the importance of CTS use across the Toronto region in comparison to the impact from more widely used asphalt materials. Over 100 samples taken across the city fell into four main categories of CTS, asphalt sealcoats (AS), asphalt, and other material. PAH concentrations in CTS ranged from 54 – 24 000 mg kg<sup>-1</sup>, in comparison to uncoated asphalt (0.1–0.9 mg/kg) and concrete areas of driveways and parking lots (0.02–0.15 mg/kg). Quinoline concentrations ranged from 0.2 to 1700 mg kg<sup>-1</sup>. We estimated that only 20 % or 14.8 km<sup>2</sup> area of a total of 630 km<sup>2</sup> impervious surface was coated in CTS. The Multimedia Urban Model (MUM) is also used to assess the main transportation mode for the PAH from the sealcoat into other environmental matrices. Results from the model indicated that the use of CTS in Toronto could contribute between 24 – 14 000 kg y<sup>-1</sup> PAH to nearshore Lake Ontario concentrations with the wide range due to uncertainties in CTS removal rates. This compares to total PAH loadings to Lake Ontario via surface waters of 35 000 kg y<sup>-1</sup>. Thus, 0.07 % – 40 % of PAH loadings from Toronto to Lake Ontario could be from CTS use.

**WP181 Is a Standard Method Desirable for Avian Egg Injections in Toxicological Assessments?** T. Augspurger, US Fish and Wildlife Service, Ecological Services; G.H. Heinz, Patuxent Wildlife Research Center, USGS, USGS, PWRC – Beltsville Lab. Injection of toxicants into the avian egg is a common approach for assessing early lifestage effects, but there is no standard method. Age of the embryo at dosing, portion of the egg receiving the dose, vehicle, vehicle volume, and incubation conditions can influence toxicological endpoints. Differences among studies in each of these aspects of experimental design limit integration of results into previous work. A standard method would facilitate comparing the relative toxicity of chemicals to a particular avian species and comparing the relative sensitivity of various bird species to a particular chemical, but standardization would best follow from objectives of the assessment. We suggest four distinct objectives which may facilitate development of one or more standard methods: 1) comparison of chemical (or chemical mixture) toxicity within a species; 2) comparison of chemical or mixture toxicity among species; 3) prediction or reproducing the toxicity observed with maternal deposition of the contaminant; and 4) examination of mechanisms of embryo toxicity. For the first two objectives, development and adherence to standard methods may aid in synthesis of results among studies and increase confidence in results among labs (and therefore support regulatory acceptance of results). For the third and fourth objectives, optimizing experimental design for the specifics of the chemical and species being evaluated likely outweigh the value in applying a standard method. An experimental design framework for each of these objectives is proposed.

**WP182 Some Reflections on Egg Injections** G.H. Heinz, Patuxent Wildlife Research Center, USGS, USGS, PWRC – Beltsville Lab; T. Augspurger, US Fish and Wildlife Service. Scientists have been injecting eggs with toxic substances for decades, but there are still many questions that need to be answered in order to fully understand how applicable the results are in terms of protecting the embryonic stage of egg laying animals. This presentation addresses some of those questions, largely through our own experiences in conducting egg injection studies with many species of birds. The biggest

question one faces is whether the administration of a contaminant by way of an egg injection mimics the toxicity when the mother deposits that contaminant in her eggs? If it doesn't, can we make adjustments to the LC50s egg injection studies generate? Egg injections may work better for certain classes of contaminants than others, but at present, we don't know which ones it works best with. Even simple questions, such as where should a given contaminant be injected – into the yolk, albumen, or air cell – have not been adequately addressed. More complicated issues, such as which solvent to use, how much of the solvent to inject, and when to inject are even less studied. The same can be said for what happens at the molecular level; when a contaminant is injected does it just float around in the egg or does it bind to compounds in the egg? Normally, one might collect eggs from animals in nature to inject, but if those wild eggs contain some of the same contaminant, how do you add together the naturally bound and injected concentrations and assess their effects? When eggs are injected and incubated artificially in the lab, hatching success of the eggs, even the control eggs, can be very poor. How can this be improved to give better dose response results? Could the results from egg injection studies ever fit into the setting of regulatory guidelines? Can egg injections become an acceptable way to conduct otherwise impossibly costly interaction studies with two or more contaminants? All of these questions, and more, are deserving of discussion and research.

**WP184 Body Distribution of Mercury in the Chicken Embryo Following Methylmercury Chloride Egg Injection** J. Rukiewicz, Univ of Michigan; D. Nam, Univ of Michigan, Dept of Environmental Health Sciences; J. Head, Univ of Michigan, Cooperative Institute of Limnology and Ecosystem Research, Univ of Michigan, Cooperative Institute for Limnology and Ecosystem Research; N. Basu, Univ of Michigan, Dept of Environmental Health Sciences, Univ of Michigan, Dept of Environmental Health. Methylmercury (MeHg) is a potent environmental toxicant that is associated with decreased reproductive success in wild and captive birds. Although females deposit methylmercury into their eggs, few studies have investigated exposure and subclinical toxic effects in the developing embryo. Even fewer have characterized the toxicokinetics of mercury (Hg) deposition across organs during development. The goal of this study was to examine the body distribution of Hg in embryos exposed at the start of incubation in order to gain a better understanding of potential sites of toxicity. To mimic maternal deposition, fertilized white leghorn chicken eggs were injected at day 0 with 0, 0.17, 0.62, or 2.0 mg/g egg MeHg chloride in corn oil. Following injection, eggs were artificially incubated in the laboratory until day 19, when embryos were dissected. Samples from the brain (cerebrum, cerebellum, optic lobe), liver, heart, kidney, muscle, and feather were collected and stored at -80°C until wet tissues were analyzed on a direct mercury analyzer (DMA-80) for total Hg. Embryos in the 0 mg/g vehicle control group did not accumulate appreciable concentrations of mercury in their tissues. Regardless of Hg dose group, Hg concentrations were highest in feathers, followed by liver, kidney, muscle, heart, and brain of Hg exposed embryos. However, embryos in the 0.62 and 2.0 mg/g dose groups accumulated a higher burden of Hg in the feathers and liver (feather Hg=12-14X brain Hg; liver Hg=3-4X brain Hg) relative to the brain than did embryos in the 0.17 mg/g dose group (feather Hg=7X brain Hg; liver Hg=2X brain Hg). In all dose groups, Hg concentrations did not differ significantly between brain regions. These results indicate that chicken embryos accumulate a body distribution of Hg similar to that of adult birds, and may similarly sequester Hg in the liver and feathers to protect against toxicity in the brain.

**WP185 A Field Egg Injection Study to Evaluate Methylmercury Toxicity in Common Loons (*Gavia immer*)** K. Kenow, USGS; M.W. Meyer, Wisconsin Dept of Natural Resources; R. Rossmann, USEPA; A. Gendron-Fitzpatrick, Univ of Wisconsin; B. Gray, US Geological Survey, Biological Resources Division. To determine the level of in ovo methylmercury exposure that results in detrimental effects on fitness and survival of common loon (*Gavia immer*) embryos and hatched chicks, we adapted egg injection techniques for field use. Eggs were injected with methylmercury chloride solutions on day 4 of incubation to achieve targeted increases in egg mercury concentrations of 0.0 (control, corn oil carrier only), 0.5, 1.3, and 2.9 µg mercury/g of wet-weight egg content. Eggs were injected at a rate of 0.5 µl solution/g egg content. Eggs were collected following about 23 days of natural incubation and then artificially incubated to observe hatching. Reduced embryo survival was evident in eggs injected at a rate of ≥1.3 µg mercury/g wet-mass. When maternally deposited mercury and injected mercury were

considered together, the median lethal concentration of mercury (LC50) was estimated to be 1.78 µg mercury/g wet-mass. We also observed increased length of incubation period, differences in chick organ mass patterns, and behavioral changes (chicks less responsive to a frightening stimulus) with increasing egg mercury concentrations. The results of this work contribute to our understanding of a critical exposure component needed to assess the risk of mercury to common loons.

**WP186 Effects of an Environmentally Relevant PCB Mixture on Embryonic Heart Development in *Gallus domesticus* (Domestic Chicken)** T. Carro, Univ of Maryland, graduate student; L. Taneyhill, Univ of Maryland, Dept of Animal & Avian Sciences; M. Ottinger, Univ of Maryland, Dept of Animal and Avian Sciences. Polychlorinated biphenyls (PCBs) are synthetically made chemicals that consist of biphenyl rings with one to ten chlorine substitutions. Mixtures of PCBs were commercially used in the US from the 1920's until their ban in the 1970's and desirable due to their overall general inertness. PCBs have been linked to adverse health conditions such as carcinogenicity, endocrine disruption, reproductive interruption, and decreased neurological function. In 2007, a study conducted in a wild bird population determined that embryonic exposure to a single PCB congener did not affect survivability of the population, but did affect heart morphology in hatchlings. To examine whether PCBs adversely affected heart development in ovo, an environmentally relevant PCB mixture was administered to broiler chicken embryos. The results of this study showed that the PCB mixture decreased survivability at increasing concentrations, affected heart rate during embryonic development, and produced a variety of cardiomyopathies in hatchling hearts; including absence of the heart wall compact and trabeculated layers, enlarged ventricular cavities, and abnormal septal formation. The effects noted in this hatchling study suggested a disruption of protein essential in heart muscle formation during embryonic heart development. Among the protein candidates was ventricular myosin heavy chain (VMHC), which is a major structural component of heart muscle that is expressed throughout the entire heart field during early heart tube formation. To explore the possibility of spatial-temporal disruption of VMHC in the embryonic heart due to PCB exposure, embryos were exposed to a low and high concentration of the PCB mixture and collected at three significant stages of chick heart development. Immunohistochemistry with a VMHC antibody was used to categorize morphological abnormalities in whole mount and on sections. The results from this study will determine the stage(s) of early cardiac development most vulnerable to PCB exposure and will potentially delineate the molecular basis of underlying cardiomyopathies observed in PCB-treated hatchling hearts. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees or the United States.

**WP187 Apparent Tolerance of Common Tern (*Sterna hirundo*) Embryos to a Pentabrominated diphenyl Ether Mixture (DE-71)** R.S. Lazarus, Patuxent Wildlife Research Center, USGS; B.A. Rattner, Patuxent Wildlife Research Center, USGS, USGS-Patuxent Wildlife Research Ctr, Beltsville Laboratory; G.H. Heinz, Patuxent Wildlife Research Center, USGS, USGS, PWRC – Beltsville Lab; S.E. Warner, N.K. KarounaRenier, S.L. Schultz, Patuxent Wildlife Research Center, USGS; R.C. Hale, Virginia Institute of Marine Science, Environmental & Aquatic Animal Health. Recent polybrominated diphenyl ether (PBDE) embryotoxicity studies with American kestrels (*Falco sparverius*) suggest the lowest-observed-effect-level (LOEL) for pipping and hatching success to be 1.8 µg total pentaBDE/g ww (~32 µg/g lw). Concentrations of total PBDEs in Forster's tern (*Sterna forsteri*) eggs from the San Francisco Bay range up to 63 µg/g lw, and thus exceed this LOEL. As a surrogate for west coast terns, 60 recently laid common tern (COTE) eggs were collected from Poplar Island, MD. Six eggs (vehicle injected controls that failed to hatch) were chemically analyzed and all were found to contain low levels of organochlorine pesticides (< 0.08 µg/g ww), total PCBs (< 0.5 µg/g), and total PBDEs (< 0.05 µg/g), indicating that sample eggs from this mid-Bay location could be used for studying DE-71 embryotoxicity. Eggs were artificially incubated, and on day 4 of development, corn oil vehicle (n=19, controls), 0.2 µg/g (n=12), 2 µg/g (n=12), or 20 µg/g (n=17) of a pentaBDE mixture (DE-71) was injected into the air cell at a constant volume (0.5 µl/g egg). There were no significant effects of DE-71 on survival to 90% of incubation (DE-71: 63.6-90.9% vs. 87.5% for controls), to pipping (63.6-81.8% vs. 81.8%), and to hatching (63.6-75.0% vs. 81.3%). Controls hatched on average 0.44 days earlier than the DE-71



groups ( $p < 0.015$ ). No gross deformities were observed, and the lengths of the tibiotarsus, metatarsus, femur, humerus, and ulna in cleared skeletal preparations did not differ among groups. Crown-rump lengths in the 0.2 and 2  $\mu\text{g/g}$  treatments were greater ( $p < 0.01$ ); however, the magnitude of change did not seem to be biologically significant. Weights of hatchlings, yolk sac, liver, bursa, thyroids, and organ to body weight ratios were not affected by DE-71, although the spleen:body weight ratio of the 2  $\mu\text{g/g}$  group differed ( $p = 0.03$ ) from other groups. Histopathological examinations of liver, kidney, spleen, bursa of Fabricius, and thyroid were unremarkable. Oxidative stress and genotoxicity assays are being conducted on liver tissue. These results suggest that COTE embryos, and perhaps other tern species, are no more sensitive (and probably less sensitive) to PBDEs than kestrel embryos. A similar pattern of sensitivity between terns and kestrels has been observed in egg injection studies involving PCB-126 and methylmercury.

**WP188 Gonadal Structure and Development of Turtle *Malayemys Macrocephala* After Topical Application of Environmentally Relevant Dose of Cadmium to Eggshell** N. Kitana, Chulalongkorn Univ, Dept of Biology; S. Keithmalesatti, Khon Kaen Univ, Dept of Environmental Sciences; M. Chiewchanchai, J. Kitana, K. Thirakhupt, Chulalongkorn Univ, Dept of Biology, Faculty of Science. In Thailand, cadmium (Cd) contamination at Mae Tao river basin, Tak Province raise concerns over potential impacts to human. The contamination is so severe that cadmium can be found in sediments, soil and grains of rice that grew in that area. However, there is still limited information on impacts on biological systems, especially animals living in the area. As a result, the monitoring system for potential effects on human populations is thus unlikely. In this study, a freshwater turtle, *Malayemys macrocephala*, was used as a sentinel species to monitor the effects of exposure and, by inference, the potential for human health impacts. Since contaminant may affect turtle by exposing through eggshell as well as maternal transfer via yolk, effect of cadmium on turtle embryos was thus examined. Freshly laid eggs were collected from a reference site with no history of Cd contamination. Representatives of eggs were subject to an ICP/ES analysis to verify that the Cd content in egg yolk is non-detectable. The effect of cadmium was determined in eggs subjected to topical exposure to  $\text{CdCl}_2$ . The doses used (0, 3, 30 and 300  $\mu\text{g}$  total Cd/g egg weight) were based on Cd content in soil at Mae Tao areas (3.4-284  $\mu\text{g/g}$ ). Eggs were kept in an incubator at 29°C, the temperature that yields 1:1 sex ratio, until hatch. Cadmium content carried across the eggshell was estimated by GF/AAS analysis in different egg compartments two weeks after exposure. It was found that 0.07% of the applied dose could be transferred to egg yolk. Hatching success (31.43%-48.57%) and hatchling weight (6.11-6.55 g) were not significantly different among groups. However, low dose of cadmium significantly prolonged the incubation time compared to those of the control (147 vs. 113 days). Sex ratio of hatchlings from the control showed 1:1 sex ratio while the increasing dose of cadmium produced the male-biased sex ratio, although the  $G$  test did not show significant difference from expected ratio (1:1) in any groups. Gonadal structure of neonate turtle was examined histologically, and the results will be presented. Potential interference of cadmium with gonadal structure and development of the freshwater turtle after topical exposure through eggshell will be discussed.

**WP189 Estrogenic Activity Assessment of Propylparaben in Water During Photochemical Degradation** T. An, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry; H. Fang, Graduate School of Chinese Academy of Sciences; G. Li, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry; X. Nie, Institute of Hydrobiology, Jinan Univ. Pharmaceutical and personal care products (PPCPs) have recently been classified as pollutants because of their ubiquitous detection in surface water and water treatment systems. One of the widely used PPCPs is propylparaben, which is applied as preservative in products such as cosmetics and shampoo. There are many references that have reported the estrogenic activity of propylparaben, which has raised a concern about its potential impact on environment and human health. However, the information on the environmental fate of propylparaben residues is limited. Thus, its environmental fate and effect on the organisms are of great concern. Photochemical degradation is an efficient process for most of PPCPs. Organic compounds could be mineralized through a series of complex reactive ways, such as reacting with  $\bullet\text{OH}$ ,  $\text{IO}_2$ , and the triplet state of compound itself or other sensitizers. Thus, in this work, photochemical degradation of propylparaben in water was studied in detail. It shown that degradation rate

of propylparaben decreased with the increasing of initial pH. To better know the role of different reactive oxygen species (ROs) during photochemical degradation, different specific scavengers were used. Results indicated that triplet state of propylparaben had the major contribution in the degradation of propylparaben in aqueous solution. In addition, the transient spectrum of triplet state of propylparaben was examined to probe the degradation mechanism. The photochemical degradation mechanism of propylparaben was tentatively proposed based on several identified degradation intermediates by LC/MS/MS. Furthermore, the change of toxicity and estrogenic activity during the photochemical degradation were also attempted. Acknowledgments: Financial supports from Knowledge Innovation Program of CAS (No. KZCX2-YW-QN103) and NSFC (No. 40973068).

**WP190 Is This Dirt Hazardous? Identification and Differentiation of Contamination Sources in the Environment** C. Yang, Z. Wang, Z. Yang, B. Hollebone, C. Brown, Environment Canada. Petroleum-related contamination is one of the most widespread environmental issues in Canada, North America, and worldwide. Remediation of the contaminated sites to meet the regulatory standards for agricultural, commercial and residential uses is usually very costly. Ubiquitous organic contaminants in the environment are generally originated from three main sources, i.e., petrogenic (PHCs), pyrogenic and biogenic (BOC). The naturally biogenic organics in soils and sediments can be easily misidentified and quantified as regulated hazardous petroleum hydrocarbons if not properly determined. In some cases, these non- or low hazardous biogenic interferences can exceed regulatory levels, resulting in unnecessary remediation. The paper introduces methodologies for the characterization and differentiation of the contribution of various sources to environmental contamination. Chromatographic analysis provides fingerprints of petroleum-related hydrocarbons such as alkylated PAHs (APAHs) and biomarkers, and biogenic compounds (BOCs) such as waxes, sterols, fatty acids and fatty alcohols. Because biomarker compounds including bicyclic sesquiterpanes and diamondoids are naturally absent from modern soils and sediments that are free from petroleum contamination, in addition to the signatures of determined APAHs and traditional biomarker terpanes and steranes, their presence provides further evidence of petroleum contamination. A series of diagnostic ratios are found to be effective in distinguishing biogenic source from others in environmental samples.

**WP191 Fate of Airborne Cyclic Organic Siloxanes in the Presence of Ozone and UV Lights and Its Implications** Z. Gao, Health Canada, Exposure and Biomonitoring Division, Healthy Environments and Consumer Safety Branch; Y. Feng, J. Zhu, Health Canada; J. Zhang, Syracuse Univ, Dept of Mechanical and Aerospace Engineering. The objective of this study is to investigate the effects of environmental factors (ozone and UV light) on fate of airborne cyclic organic siloxanes, and potential formation of secondary compounds. Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) were selected for the study. The tests were carried out in a full-scale stainless steel environmental chamber with an interior volume of 56.4 m<sup>3</sup>. The chamber air conditions were set at 23°C ( $\pm$  2°C) and 50% ( $\pm$  10%) RH. Four UVA and four UVB lamps were placed at the corners in the chamber. For UVA lamps, the max light intensity at the lamp surface was 20.5 mW/cm<sup>2</sup> by UVA meter and 0.12 mW/cm<sup>2</sup> by UVB meter. For UVB lamps, the max light intensity at the lamp surface was 5.5 mW/cm<sup>2</sup> by UVA meter and 2.48 mW/cm<sup>2</sup> by UVB meter. The light intensity decreased below 1 mW/cm<sup>2</sup> for both UVA and UVB at 1 m away from the lamp. Ozone was generated by an ozone generator and its concentration was monitored continuously by an ozone analyzer. D4 or D5 was spiked into the chamber to create a concentration of 1 mg/m<sup>3</sup>. Formaldehyde concentration was monitored by 1412 gas monitor while other VOCs (including D4 or D5) were analyzed by GC/MS. Significant ozone consumption accompanied with measurable formaldehyde production was observed when D4 (and D5) was mixed with ozone in the presence of UV lights. A clear decrease of D4 (and D5) concentration in the chamber was also observed when the compound was exposed to a combination of UV light and ozone. D4 and D5 were however, found to be stable in the presence of either ozone or UV light alone. It appears that UV light promoted the production of ozone in the chamber thereby promoted reduction of airborne siloxanes. The identification and quantification of reaction products need to be further investigated.

**WP192 Alternate Approaches to Assessment of Benthic Health in Sediment Limited Systems** W.J. Reese, J.R. Flanders, URS Corporation;

C. Mancini, URS Corporation, Manager-Ecosystems Management Team; R.G. Stahl, DuPont Company, Corporate Remediation Dept, Principal Consultant, DuPont Corporate Remediation Group. Benthic macroinvertebrate community structure has been used as to measure habitat integrity and the effect of environmental stressors over time in a variety of regulatory and environmental settings. The benthic macroinvertebrates inhabiting fluvial systems include a range of trophic levels, providing information for potential cumulative effects of stressors as well as a detailed understanding of the benthic macroinvertebrate component of the aquatic food web. In order to assess benthic community structure and colonization dynamics along a gradient of mercury, a benthic colonization study was designed and implemented in the South River, Virginia, which has elevated levels of mercury compared with regional background levels due to historic uses of mercury in the watershed. The study design included deployment of uniform substrate types, standardized deployment parameters, and controlled exposure times to minimize variability between treatments. Substrate-filled colonization trays were deployed in riffles at four study sites downstream of the historic point source of mercury and two reference sites, and will be sampled after 14, 28, and 42-day colonization periods. Co-located benthic samples will also be collected using a Surber sampler to characterize resident benthic community structure by means of a traditional method. Additionally, co-located interstitial sediment samples will be collected and analyzed for total mercury (THg) and methylmercury (MeHg) to establish potential concentration gradients in physical media. Specific community metrics will be evaluated to characterize the colonized and resident community samples. Statistical analyses may include analysis of variance (ANOVA) and correspondence analysis to evaluate differences in benthic colonization community structure in the South River, Virginia.

**WP193 Arsenic Speciation and Biotransformation in Highly Contaminated Marine Benthic Macroinvertebrates** K. Whaley-Martin, I. Koch,

K. Reimer, Royal Military College of Canada, Environmental Sciences Group. The ability to predict and mitigate toxic effects of arsenic in marine environments is dependent on knowledge of how arsenic is biochemically transformed and the factors controlling these mechanisms. Arsenobetaine, a non-toxic arsenic compound, is commonly found in marine animals, and recent studies point to the possibility that the formation or uptake of this compound is compromised in organisms exposed to high concentrations of arsenic in severely contaminated environments. In such environments, we hypothesize that arsenic is taken up unchanged, as toxic inorganic arsenic. The first part of this investigation measured the proportion of inorganic arsenic, using complementary techniques (high performance liquid chromatography – inductively coupled plasma mass spectrometry and x-ray absorption near edge structure analysis) in three common marine benthic macroinvertebrates (Blue Mussels (*Mytilus edulis*), Edible Periwinkles (*Littorina littorea*) and Shore Crabs (*Carcinus maenas*)) as a function of an arsenic concentration gradient. Preliminary results show strong positive correlations between the increase in the proportion of inorganic arsenic and the increase in total arsenic present in all three marine benthic organisms. This may have significant implications with respect the health of marine biota and humans consuming them. The second part of this investigation examined the bioaccumulation and metabolism of arsenic more closely in *Mytilus edulis* by in situ elemental mapping the arsenic distribution in a fully intact organism from a contaminated site. The gills, mantle and digestive gland, which are organs that are important for analyte absorption and uptake, were targeted. Mapping was carried out at the known k-edge absorption photon energies of three arsenic species: As(V) (11875.3 eV), AB (11872.6 eV) and As(III) (11871.7 eV), as well as at 11868 eV for total As, Se, Br, Zn, Cu, Ni, Fe, Mn, Ti, Ca, Cr, K, Hg, S and P mapping. Preliminary results revealed interesting As(III) and As-S hotspots in the digestive gland which do not seem to correlate with any other elements, indicating the hot spots are biological and not mineralogical.

**WP194 Dietary Arsenic Toxicity in Subadult Rainbow Trout: Growth Effects, Nutrient Absorption, and Tissue Bioaccumulation** D. Hoff, T.L.

Highland, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; J.R. Hockett, USEPA, Mid-Continent Ecology Division; C. Jensen, M. Poe, USEPA. Dietary arsenic toxicity in subadult (~200 g.) rainbow trout was evaluated in a 70 day test using arsenic-spiked pellet diets containing 50, 104 and 162 ppm arsenite. All organisms in all treatments survived the exposure. Dose dependent effects on percent weight gain, with commensurate changes in feed conversion and efficiency, were

noted in all arsenic-spiked diets. Growth, as measured by standard length, was similarly affected in a dose-dependent manner. Chromic oxide was also spiked into all pellet diets at a concentration of 0.3% and was used as a marker for measuring absorption of gross lipid and protein in the contaminated diets. Weekly fecal samples were collected to detect changes in nutrient digestion throughout the 10 week exposure. Little to no change in protein absorption was noted throughout the exposure period. While lipid absorption was similar for the first 4 weeks across all treatments (91-94%), they subsequently decreased progressively in arsenite-spiked treatments through the end of the experiment to as low as 80% in the higher doses. Liver, kidney, pyloric caecum, gall bladder and muscle tissue were harvested at the termination of the study allowing for the calculation of tissue-specific bioaccumulation factors. Findings are discussed in the context of previous studies of arsenic effects and the potential for developing residue-based assessment approaches. This abstract does not necessarily reflect EPA policy.

**WP195 Evaluating the Effects of Sub-lethal Copper Exposure on Ammonia Excretion in the Euryhaline Guppy, *Poecilia vivipara*** A.M. Zimmer, McMaster Univ; I.F. Barcaroli, Universidade Federal do Rio

Grande – FURG; C. Wood, McMaster Univ; A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. Copper is a common toxic pollutant in aquatic environments and though Cu is known to be lethal at elevated levels, the physiological effects of non-lethal Cu exposure are not well understood. To date, studies seem to suggest that Cu exposure disrupts Na<sup>+</sup> uptake and ammonia excretion (J<sub>amm</sub>). These observations are very interesting physiologically as these processes are thought to be linked to one another in the current molecular model for ammonia excretion. Acute 96 h exposure to 20 µg/l Cu using *Poecilia vivipara*, a euryhaline guppy collected at a creek flowing into the sea at Cassino Beach (Southern Brazil), demonstrated that fish acclimated to saltwater (SW; 25ppt) are more sensitive than those acclimated to freshwater (FW; 0 ppt). Though Cu exposure elicited a transient inhibition of J<sub>amm</sub> in both FW- and SW-acclimated fish, the exposure resulted in a reversal of J<sub>amm</sub> in the SW-acclimated fish. However, following 84 h of Cu exposure, both acclimation groups were able to recover J<sub>amm</sub> to control levels. To further understand the mechanism of the compensatory responses displayed by both fish groups, 24 h and terminal activity of carbonic anhydrase (CA), Na<sup>+</sup>/K<sup>+</sup>-ATPase, and H<sup>+</sup>-ATPase will be measured to assess if these enzymes play a role in the observed inhibition and recovery of J<sub>amm</sub>. Furthermore, the effect of copper on Na<sup>+</sup> homeostasis will be assessed by measurements of terminal whole-body [Na<sup>+</sup>] in both fish groups. The ultimate goal is to develop a model for understanding the physiological effects of non-lethal Cu exposure in both SW- and FW-acclimated fish. [Supported by the International Research Chair Program of the International Development Research Centre, the Canada Research Chair Program and the Brazilian CNPq (INCT-TA)].

**WP196 Investigation of Arsenic Speciation in Plankton Organisms from Contaminated Lakes by HPLC-ICP-MS and XAS** G. Caumette,

I. Koch, K. Reimer, Royal Military College of Canada, Environmental Sciences Group. Arsenic is mainly found as a toxic inorganic compound in water, while aquatic organisms contain organoarsenicals, with the non-toxic arsenobetaine being the main compound found in marine organisms. For health and safety reasons, such as the exposure of local populations, there is still a need to understand the cycling of arsenic through the food chain, and the pathways of formation of the non-toxic arsenobetaine. The marine environment has been widely studied and arsenobetaine is found as the major arsenic compound in most animals, including zooplankton, which is at a low trophic level of the marine food chain. Marine algae and phytoplankton, on the other hand, contain mainly arsenosugars. A hypothesis to explain these findings describes arsenobetaine as an osmolyte needed in saline environments (Clowes et al. 2004). The present work studies arsenic speciation at the base of the freshwater food chain, in plankton. Although the osmolyte requirements are not the same, arsenobetaine has been found in freshwater fish (Miyashita et al. 2009). Phytoplankton and zooplankton species were collected from gold-mine contaminated lakes in Yellowknife, NT, Canada. Arsenic speciation was measured in phytoplankton and the different zooplankton species isolated (*Cyclop* sp. and *Daphnia pulex*). HPLC-ICP MS (high performance liquid chromatography – inductively coupled plasma mass spectrometry) was used on water-extracts and XAS (X-ray absorption spectroscopy) was used on the whole solid samples and residues from extraction, in order to assess speciation of the unextracted arsenic. The results show that unlike marine phytoplankton (which contain



arsenosugars), freshwater phytoplankton only contain inorganic AsV. However freshwater zooplankton contain several organoarsenicals, with the sulphate arsenosugar as the most abundant, up to 47% of total arsenic. The other arsenic compounds identified were the phosphate arsenosugar, the glycerol arsenosugar, MMA (monomethylarsonic acid) and DMA (dimethylarsinic acid). The proportion of these compounds in zooplankton organisms varies according to the species analyzed and the arsenic concentration in the lake. In uncontaminated lakes, arsenobetaine was found in *Cyclop* sp. as a minor compound, less than 8% of total arsenic measured but it was not present in organisms from contaminated lakes. This information supports the likelihood that arsenobetaine is not required, by freshwater organisms as much as in the marine environment.

**WP197 Metal Accumulation from Dietary Intake in the Sea Urchin, *Strongylocentrotus droebachiensis*** T. Jarvis, Valdosta State Univ, Biology; G.K. Bielmyer, Valdosta State Univ, Biology; Valdosta State Univ, Dept of Biology. Heavy metal contamination is an increasing problem in aquatic environments; however, little research has focused on the effects of dietary metal exposure to macroinvertebrates. The seaweeds, *Ulva lactuca* and *Enteromorpha prolifera*, readily uptake metals, are widely distributed, and are often used as bioindicators in ecological studies. Additionally, these species serve as vital components in aquatic food chains. *U. lactuca* and *E. prolifera* were exposed to five metals (Cu, Ni, Pb, Cd, and Zn) and fed to the green sea urchin, *Strongylocentrotus droebachiensis*, for two weeks. Body mass, test length, and total length were measured, the sea urchins were dissected and their organs (esophagus, stomach, intestine, gonads, and rectum) were digested and analyzed for metal content using graphite furnace atomic absorption spectrophotometry. The results demonstrated that metal accumulation and distribution varied between seaweed species and among metals. All of the metals accumulated within at least one organ of *S. droebachiensis*, with Cu being most significant. In general, there were higher levels of metals within the sea urchins fed *E. prolifera* as compared to those fed *U. lactuca*. These results indicate that *E. prolifera* may accumulate metals in a more bioavailable form than within *U. lactuca*, which may substantial impact metal toxicity in the consumer. No significant differences in body length or growth were detected between the control and metal-exposed sea urchins after the two week testing period, despite the observed increase in tissue metal accumulation. However, increased exposure duration may lead to more substantial effects.

**WP198 A Multi-media Approach to Stream Metal Contamination in Canada's "Chemical Valley": Implications for the Aamjiwnaang First Nation** D. Cryderman, L. Letourneau, N. Basu, School of Public Health, Univ of Michigan, Environmental Health Sciences. Approximately 40% of Canada's petrochemical industry lies near the Aamjiwnaang First Nation Reserve, in Sarnia, Ontario. Previous work has shown the possibility that various metals, among other pollutants, in a local stream may pose threat to wildlife species and human residents. Here we initiate a broader study (spatially and temporally) to address the levels of multiple metals (e.g., cadmium, cobalt, lead, manganese, and zinc) in stream water on and near the Reserve, as well as mercury levels in sediment and soil collected from those streams. The purpose was to determine if sites on Reserve have higher concentrations of these metals than off Reserve, and to identify any site at which levels exceed benchmark values. Surface water, sediment and soil were collected from twenty-five stream sites were analyzed for metals during the fall of 2010 and spring of 2011. Three out of twenty-five stream sites showed elevated levels of lead (ranging from below LOD to 379 ppb), one site had elevated levels of cobalt (ranging from below LOD to 7 ppb), and four sites had elevated manganese levels (ranging from 4ppb to 1924ppb) in stream water. In general, stream water concentrations of metals were higher in the fall than the spring. Levels of mercury in sediment (0.2ppb-1830ppb) and soil (1.2 ppb – 646ppb) collected near sites were elevated in multiple streams, with concentrations in the fall tending to be higher than in the spring. Overall, these findings suggest that metals contamination is ubiquitous in the region, and that spatial and temporal variation exists. Future work is needed to determine the bioavailability (and possible health effects) in resident wildlife and humans.

**WP199 Benthic Macroinvertebrates Community and Sediment Toxicity in a Metropolitan Water Supply Reservoir in Brazil** E. Lage, A.L. Brandimarte, Universidade de São Paulo – USP, Dept of Ecology; C.M. Botta, E.L. Espindola, Universidade de São Paulo – USP, Escola de

Engenharia de São Carlos – EESC. The high demand for water supply led Guarapiranga reservoir, built primarily for power generation, became a relevant public water supply to Sao Paulo city (Brazil) since 1928. Moreover, due to high urbanization, nowadays more than half of its basin area is modified, sewage and diffuse pollution are the main pollution sources. The relationship between benthic community and sediment features was evaluated in three sites, in each one of four sampling stations (one near the dam and three in the main reservoir tributaries: Embu-Mirim, Embu-Guacu and Parelheiros). In July 2010, 6 sampling units for benthic fauna analysis and 3 for sediment bioassays were collected with an Ekman-Birge grab in each sampling site. Fauna samples were washed into a 500 µm mesh sieve and preserved in 4% neutralized formalin in the field. In the laboratory, samples were transferred to 70% alcohol and stained (Rose Bengal). Two sediment samples of each site were collected with an Ambühl & Bühler corer for physical and chemical measures. All sites had 7 taxa, except Embu-Guacu with 9. Oligochaeta was the most abundant group in all the sites, except in Parelheiros where Chaoboridae was most abundant. Silty sediment predominated, except in Embu-Guacu where was a sandy site. The average organic matter sediment content was 35%, with higher value in the dam region. Extremely reducing conditions were observed in Embu-Mirim and near the dam, especially in the drinking water catchment area. Another author found the highest values of the relationship ( $\Sigma$  [SEM] – [AVS]), TOC, the highest concentrations of metals and concentrations above PEL for chromium, copper and lead, and above VRR for all metals analyzed, except nickel around the dam. Presence of Oligochaeta and Ostracoda was associated with the organic matter content and presence of silt. Besides Chaoboridae, other insect larvae were found, including Tanypodinae, *Chironomus decorus* and Ceratopogonidae. The test with *Daphnia similis* showed significant sediment acute toxicity in all regions except in Embu Guacu. The test with *Chironomus xanthus* showed significant sediment acute toxicity in all regions except for one site in Embu Guacu and one in Embu-Mirim. The test for porewater with *Daphnia similis* showed significant acute toxicity in the dam region. Spatial heterogeneity, due to many of the variables analyzed, divided Guarapiranga reservoir in 2 compartments: Embu Guacu and Parelheiros; Guarapiranga-dam and Embu-Mirim.

**WP200 Can Freshwater Toxicity Models (FIAM and BLM) Be Applicable to Marine Ecosystems?** Y. Dong, Denmark Technical Univ, Section of Quantitative Sustainability Assessment, DTU Management Engineering; M. Owsianiak, Technical Univ of Denmark, Dept of Management Engineering; K.S. Christiansen, Univ of Copenhagen, Dept of Basic Sciences and Environment, Faculty of Life Sciences, Denmark Technical Univ, Section of Quantitative Sustainability Assessment, DTU Management Engineering; M.Z. Hauschild, Denmark Technical Univ, Section of Quantitative Sustainability Assessment, DTU Management Engineering. Metal toxicity in freshwater ecosystems is relatively well documented and predictive models such as biotic ligand models (BLM) or free ion activity models (FIAM) are available. However, toxicity of metals in marine and estuarine waters has attracted less attention, despite the fact that these waters receive a large share of total metal emission from industrial activities. While recent studies report that metal uptake rates for marine fish are comparable to those for freshwater fish, they also reported on higher sensitivities of freshwater organisms to toxic metals when compared to marine species. We hypothesize that the observed differences are a result of differences in water compositions, with elevated concentrations of cations in marine waters reducing metal toxicity by competitive binding to biotic ligands, and the presence of anions to form complexes with toxic metal ions. If this hypothesis is true, it should be possible to extrapolate ecotoxicity models developed for freshwater organisms to marine ecosystems by taking differences in ionic composition into account. The aim of this work is to test the applicability of freshwater FIAMs and BLMs for predicting metal toxicity in marine and estuarine ecosystems. Firstly, published BLMs for freshwater fish are employed to calculate the activity of free metal ion corresponding to EC50 in freshwaters spanning a wide range of water characteristics. Results show that toxicity of metals to fathead minnow varies about 1 order of when expressed as free ion concentrations ( $4.9\text{E-}09$ – $7.8\text{E-}08$  mol/l for  $\text{Cu}^{2+}$ ). The predictions made with FIAM fall within the range of values predicted by BLMs. Next, assuming that intrinsic sensitivity of marine fish to toxic metal is similar to that of freshwater fish, freshwater BLMs are employed to predict EC50 of metals in salt waters. The calculated free ion EC50 values are up to two orders of magnitude higher than in freshwaters. To test the accuracy of the predictions, FIAMs for marine fish are derived from published EC50 values for salt



waters by means of speciation modeling. The results show a good correlation between freshwater BLMs extrapolated to marine ecosystem, and marine FIAMs. This highlights the importance of taking metal speciation and water composition into account in predicting metal toxicity. It also suggests that existing toxicity parameters for freshwater organisms can be extrapolated to marine ecosystems.

**WP201 Chromium(III) Exposure is Toxic to Developing Zebrafish Altering Expression of Genes Critical to Matrix Metabolism (MMP) and Metal Detoxification (MT)** A.A. Rucki, K.R. Cooper, L.A. White, Rutgers, the State Univ of New Jersey, Dept of Biochemistry and Microbiology. We hypothesize that chromium (III) is a potential teratogen that exerts its effects through alteration of matrix metabolism. Furthermore, we hypothesize that metallothionein can be used as a marker for aquatic contamination with chromium (III). Chromium (III) is an essential element required for fat and protein utilization; however, at higher concentrations it can be toxic. Approximately 2,300 metric tons of chromium (III) are released to the atmosphere in the United States from a variety of sources (leather tanning, wood preserving) each year. This released chromium does not remain in the atmosphere, but is deposited into soil and water resulting in exposure of aquatic species. We exposed zebrafish embryos to chromium (III) from 2 hour post fertilization (hpf) at concentrations ranging from 0.2  $\mu$ M to 5.0  $\mu$ M, and embryos were observed at 24, 48, and 72 hpf. We found that chromium (III) exposed zebrafish showed a statistically significant increase in mortality at 2  $\mu$ M, as well as increased incidence of hemorrhaging, edema, tail curvature and yolk sack dysmorphia at 1  $\mu$ M chromium. To determine whether these changes may relate to disruption in matrix metabolism, we determined whether chromium exposure altered expression of the matrix metalloproteinases (MMPs), endopeptidases that are critical for extracellular matrix remodeling. Our data, using real time RT-PCR to analyze MMP gene expression, show that chromium exposure results in an increase in the expression of MMP-2, MMP-9, and MMP-13 at 0.2  $\mu$ M and 2  $\mu$ M, suggesting that some of the developmental effects of chromium (III) exposure may be through alterations in MMP expression. We also examined whether metallothionein could be used as markers for chromium (III) exposure. Metallothioneins (MT's) are a family of highly conserved, low molecular weight, cysteine-rich, metal binding proteins, with critical roles in heavy metal detoxification and protection against reactive oxygen species (ROS). For this study we focused on MT-1 and MT-2 and found that exposure as low as 0.2  $\mu$ M chromium (III) (ten times below the current acceptable limit in water) resulted in a significant increase in MT-1/-2 expression in developing zebrafish.

**WP202 Copper Effect on Energy Metabolism in the Sea Anemone *Actinia bermudensis*** N.L. Yano, M.M. Lauer, A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. Copper is an essential metal, but excessive concentrations in water can be toxic to aquatic animals. In general, its mechanism of toxicity in fresh water species is associated with ionic and osmoregulatory disturbances. However, little is known about its effect in marine osmoconformers like echinoderms and cnidarians. Therefore, the aim of the present study was to evaluate the effect of copper on energy metabolism in the sea anemone *Actinia bermudensis*. Animals were exposed (96 h) to copper (5, 10, and 50  $\mu$ g Cu/L) in seawater (30 ppt). Temperature (20°C) and photoperiod (12h L: 12h D) were fixed. A control test (no copper addition) was also run. No mortality was observed. Following copper exposure, sea anemones were cryoanesthetized and dissected for enzymatic analysis [hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), and citrate synthase (CS)]. Control animals showed reduced HK activity respect to those exposed to 5  $\mu$ g Cu/L. PFK activity was higher in sea anemones exposed to copper than in those kept under control conditions. On the other hand, sea anemones exposed to 5  $\mu$ g Cu/L showed a higher PK activity than the control ones, while those exposed to 50  $\mu$ g Cu/L showed an inhibited enzyme activity. CS activity was also inhibited in all copper-exposed sea anemones when compared to control ones. This copper-induced impairment may lead to a disruption of the energy metabolism in the sea anemone, since a smaller production of citrate leads to a lower production of NADH and FADH<sub>2</sub>, the co-enzymes responsible for ATP production. Our results show that even low copper concentrations (5  $\mu$ g Cu/L) may impair sea anemones physiology, suggesting that other species from neglected phyla such as Cnidaria should be used to establish safe levels of copper in marine environments. [Supported by Brazilian CNPq

(INCT-TA) and CAPES (Ciências do Mar) and Canadian International Development Research Centre (IDRC)].

**WP203 Copper Effects on Energy Metabolism in the Sea Cucumber *Trachythyone crassipeda*** M. Lauer, C. Oliveira, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas; R. Cagnin, B. Cantarella, L. Fernandes, Universidade Federal do Espírito Santo; A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. Marine organisms have being neglected about metals effects since it is believed that sea water chemistry provides a high protection against these pollutants. However, recent studies showed that they are indeed very susceptible to metal exposure. Sea cucumbers belong to an exclusive marine phylum, the Echinodermata, and very little is known about metal effect on their metabolism. Copper is usually considered an iono-osmoregulatory toxicant to aquatic animals. Since echinoderms are osmoconformers another mechanism (respiratory or energetic impairment) could be involved in copper toxicity. In light of the above, the aim of this study was to evaluate the effects of copper exposure on the energy metabolism of *Trachythyone crassipeda*, a sea cucumber found in Brazilian marine waters. Adult animals were exposed to 5, 9, and 20  $\mu$ g Cu/L for 96h at 30 ppt under fixed temperature (24°C) and photoperiod (12h L: 12h D). A control test (no copper addition) was also run. No mortality was observed during test period. After exposure, sea cucumbers were cryoanesthetized and their respiratory trees were dissected and collected for enzymatic analyses [hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), lactate dehydrogenase (LDH), citrate synthase (CS)]. HK activity was higher in sea cucumbers exposed to 9 and 20  $\mu$ g Cu/L than in control animals and those exposed to 5  $\mu$ g Cu/L. No difference in PFK and CS activities were observed between control and copper-exposed animals. PK activity was lower in control animals than in those exposed to copper. However, organisms exposed to 20  $\mu$ g Cu/L showed higher PK activity than those exposed to 5 and 9  $\mu$ g Cu/L. In contrast, LDH activity was higher in control animals, with sea cucumbers exposed to 5 and 20  $\mu$ g Cu/L showing higher activities than those exposed to 9  $\mu$ g Cu/L. Even though HK and PK are more active, neither LDH nor CS is raising its activity, which could imply in a reduction of NADH and FADH<sub>2</sub> production, and a consequently smaller ATP production. Taken altogether, these findings show that sea cucumbers rely on an anaerobic metabolism that is compromised by copper exposure since LDH, a key enzyme in this process, was impaired by all copper concentrations tested. It is clear that copper affects marine organisms such as echinoderms even at low concentrations (5  $\mu$ g Cu/L). Therefore, more attention should be paid to these animals in order to establish safe levels of copper in marine environments.

**WP204 Copper Toxicity and Bioavailability to *Mytilus galloprovincialis* in Shelter Island Yacht Basin, San Diego, CA** C. Capolupo, R.S. Kaufmann, Univ of San Diego; M. Colvin, San Diego State Univ Research Foundation; G. Rosen, SPAWAR Systems Center Pacific, Environmental, SPAWAR Systems Center Pacific, Scientist; P. Earley, B. Swope, I. Rivera, SPAWAR Systems Center Pacific. Copper concentrations in bays and marinas have the potential to reach relatively high levels due in part to leaching from copper-based antifouling paints used on aquatic vessels. These high levels may or may not be toxic depending on the chemistry of the water which affects copper bioavailability. Shelter Island Yacht Basin (SIYB), a recreational marina and harbor located near the mouth of San Diego Bay, is frequently elevated for copper, but the bioavailability and toxicity of this contamination is not well understood. It is important to examine the biological effects and chemical activity of copper in order to predict potential effects of its loading on biological communities, and to make environmental decisions about copper regulation. This study assessed the temporal and spatial variation of total and dissolved copper concentration, complexation capacity, and toxicity in SIYB. Samples were collected from 15 stations at two different depths during both wet and dry seasons (March and July 2011, respectively). Samples from four of these stations were spiked with up to 10 concentrations of copper in laboratory toxicity exposures for deriving a site-specific criterion for the Basin using the EPA's water effect ratio (WER) procedure. Toxicity tests were 48-h embryo-larval development tests with the mussel *Mytilus galloprovincialis*. The results of the ambient and WER studies will be compared to the results predicted based on water chemistry alone using the marine Biotic Ligand Model. The areas at the head of SIYB had copper concentrations above the EPA's water quality criterion of 3.1  $\mu$ g/L; however, no toxicity was observed during the wet season sampling.

This result attests to the importance of including bioavailability of the metal in regulatory decision making.

**WP205 Effect of Cations on Toxicity of Ni to Duckweed (*Lemna minor*): A Mechanistic Study** Y. Gopalapillai, Univ of Guelph, School of Environmental Sciences, Natural Resources Canada, CANMET Mining and Mineral Sciences Laboratories, Natural Resources Canada, Metal and Mineral Sciences Laboratories; B. Hale, Univ of Guelph, School of Environmental Sciences; B. Vigneault, Natural Resources Canada. Major cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ) are generally known to reduce toxicity of  $\text{Ni}^{2+}$  by competing for uptake at the biotic ligand in aquatic organisms such as fish (BLM framework), while soil plant studies have shown lack of competition. In the present study, the effect of cations on toxicity of  $\{\text{Ni}^{2+}\}$  in water (i.e.,  $\text{IC}_{25\text{Ni}^{2+}}$ ) and Ni accumulated in tissue (i.e.,  $\text{IC}_{25\text{NiTiss}}$ ) were examined using the aquatic plant, *Lemna minor*. Ni accumulation kinetics was used to provide mechanistic explanations for the observed effects. The results indicate that major cations do not significantly affect  $\text{Ni}^{2+}$  toxicity in the tested range. However, kinetic analysis of Ni accumulation showed an anti-competitive effect of  $\text{Ca}^{2+}$  on  $\text{Ni}^{2+}$  accumulation ( $K_d$  and  $B_{\text{max}}$  decreased linearly).  $\text{IC}_{25\text{NiTiss}}$  increased linearly with  $[\text{Na}^+]$  (indicating reduced sensitivity of *L. minor* to  $[\text{Ni}]_{\text{Tiss}}$  in the presence of  $\text{Na}^+$ ) but was not affected for the other cations. For pH,  $\text{IC}_{25\text{Ni}^{2+}}$  decreased linearly with pH (BLM type model); unexpectedly,  $\text{IC}_{25\text{NiTiss}}$  also decreased linearly (not a BLM-type model and indicates that plant is more sensitive to Ni at higher pH). In addition, Ni accumulation kinetics did not show 'pure' competitive relationship between  $\text{H}^+$  and  $\text{Ni}^{2+}$ . Thus, increased toxicity at higher pH may be due to other physiological or chemical changes with pH (e.g., presence of Ni hydroxides/carbonates, change in availability of micronutrients). The results of the present study suggest that toxicity of Ni in aquatic plants is dominated by homeostatic control and a model based on simple competitive cation effects is not sufficient.

**WP206 Effect of Natural Organic Matter Quantity and Quality on the Acute Toxicity of Cu to the Aquatic Amphipod *Hyalella azteca*** K. Livingstone, Wilfrid Laurier Univ, Dept of Biology; S. Smith, Wilfrid Laurier Univ, Dept of Chemistry, Wilfrid Laurier Univ, Chemistry; N. Yan, York Univ; J. McGeer, Wilfrid Laurier Univ, Dept of Biology. The aim of this study is to examine the influence of NOM quantity and quality (NOM source) on acute copper toxicity and bioaccumulation in the freshwater amphipod *Hyalella azteca*. Toxicity tests (96h) were done according to standard methods with 2-9 day old organisms added to solutions of Cu (0.25-2  $\mu\text{M}$ ) and dissolved organic carbon (0-11.5 mg C/L). The pH of test solutions was  $7.1 \pm 0.1$  and temperature was maintained between 20-21  $^{\circ}\text{C}$ . Test solutions had a water hardness of 40 mg  $\text{CaCO}_3/\text{L}$  and consisted of the following salts: 0.31mM  $\text{CaCl}_2$ , 0.31mM  $\text{NaHCO}_3$ , 0.031mM  $\text{NaBr}$ , 0.0153mM  $\text{KCl}$ , and 0.0763mM  $\text{MgSO}_4$ . Test solutions were equilibrated for 24 h before initiating exposures. The  $\text{LC}_{50}$  for dissolved Cu, with no added NOM, was determined to be 1.2  $\mu\text{M}$  Cu ( $\pm 0.9 \mu\text{M}$ ). Tests designed to understand the time course of accumulation (at 0.25  $\mu\text{M}$  Cu) showed that the saturation of tissues (whole body dry weight basis) occurred quickly, at 9 h following Cu exposure. Short term exposure to Cu that the body burden sharply increased then levelled to a maximum at 1.0  $\mu\text{M}$ , demonstrating saturable uptake and suggesting a Cu-regulating mechanism. Testing with NOM sources established a clear protective relationship between NOM quantity and toxicity. For example, Brandy Lake NOM added (0-11.5 mg C/L) at various Cu concentrations indicated a DOC concentration-dependent relationship with survival, directly related to  $\text{Cu}^{2+}$ . Tests to compare the quality (based on toxicity mitigation) of NOM sources demonstrated clear differences that could be related to Cu binding capacity (measured by  $\text{Cu}^{2+}$  selective electrode) and optical (absorbance and excitation emission matrix spectroscopy) characteristics. For example, the survival of *Hyalella* in test solutions with 4  $\mu\text{M}$  Cu and 5 mg DOC/L improved from 25% with Daisy Lake NOM to 55% with Allard River NOM. This difference in protection was associated with the increased Cu binding capacity ( $B_{\text{max}}$  of 1.07 compared to 1.61  $\mu\text{M}$  Cu/mg C), and the increased humic-like content of Allard River (56% compared to 82%). Currently the Biotic Ligand Model (BLM) includes NOM as a single homogeneous entity however; sources vary in their protective abilities. This research provides some progress toward understanding NOM quality characteristics which may be used to improve BLM predictions of Cu impacts in fresh water. This project is part of the TALER (Terrestrial Aquatic Linkages for Ecosystem Recovery) Research Program supported by NSERC, Vale and Xstrata.

**WP207 Effects of Copper on Sodium Content in Mitochondria-rich Cells of the Freshwater Mussel *Lasmigona costata*** L. Nogueira, Federal Univ of Rio Grande – FURG, Instituto de Ciências Biológicas; A. Bianchini, Federal Univ of Rio Grande – FURG; C.M. Wood, McMaster Univ; P.L. Gillis, Environment Canada – Canada Center for Inland Islands. Gill tissues in aquatic animals are composed of various cell types, each with specific functions such as respiration, excretion, acid-base balance and ionic regulation. Among the gill cells, mitochondria-rich cells (MRCs) are considered to be the primary sites responsible for ion transport. In bivalves, the gill is the main route of contaminant uptake and metals can be absorbed through specific transporters in cell membranes. In order to determine the effects of copper on sodium content in MRCs, gill cells from *Lasmigona costata* were isolated and layered in a discontinuous Percoll gradient. The pavement cells (PVCs) and MRC populations were confirmed by a specific mitochondrial dye (DASPEI) and Na,K-ATPase activity. MRCs were then exposed to a range of copper concentrations (5, 10 and 20  $\mu\text{g/L}$ ) for 3 hours. Following exposure, copper accumulation and sodium content were measured. Copper accumulation increased in all copper exposures with saturation-type kinetics. In contrast, the sodium content did not change in low exposures and increased significantly when cells were exposed to high copper concentrations. To further explore this result, both sodium and copper were measured after specific pharmacological blockade of Na,K-ATPase and carbonic anhydrase enzymes. Carbonic anhydrase inhibition had no effect, suggesting that sodium and copper uptake mechanisms are not dependent on a supply of protons. However, the inhibition of Na,K-ATPase by ouabain resulted in an increase in sodium and copper content in the MRCs. This enzyme is responsible for sodium extrusion from the cell, and the results suggest that it is also a route for copper extrusion. (Supported by the International Research Chair Program of the International Development Research Centre and the Canada Research Chair Program).

**WP208 Environmental Causes and Phenotypic Consequences of Gene Copy Number Variation: A Metal Perspective** J.R. Shaw, Indiana Univ, The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics, Dartmouth College, Dept of Biology; S.P. Glaholt, R. Keith, Indiana Univ, The School of Public and Environmental Affairs. Recent studies indicate copy number variation (CNV) represents a large source of the genetic variation observed in human populations and have uncovered strong associations between CNV and disease, including complex phenotypes. However, the environmental contributions to CNV remain unknown, in part because there are few animal models available for environmental genomics studies, which seek to understand how genome structure and function evolve in response to environmental change. Our group makes use of the NIH model, *Daphnia*, to test the central hypothesis that exposure to environmental contaminants increase the rate of mutations giving rise to CNV, and that this variation has functional consequences on gene expression, phenotype, fitness, and population structure. To accomplish this goal we are developing mutation accumulation (MA) lines derived in the absence and presence of cadmium in order to define the spectra of CNV and measure the per generation rate at which they spontaneously arise in individuals. We are studying past populations that have been captured and preserved in lake sediments. Here we scan genomes from populations that have adapted to over a century of mining pollution in order to characterize the magnitude, distribution, functional consequences, and evolutionary path of CNV in relation to the metal adapted phenotype. Finally, we are conducting quantitative trait loci experiments to determine the functional significance of CNV by establishing cause and effect relationships between copy number variants and metal tolerance. Collectively, these studies quantitatively assess whether environmental exposure affects the risk for spontaneous CNV, and do so in context of their contributions to individual health parameters that influence tolerance (i.e., adaptation and susceptibility) and disease. Answers to these questions have profound implications for the long-term health of human populations that are living longer and doing so in the presence of a greater diversity of chemicals that can modify DNA.

**WP209 Estimating Population-level HC5 for Copper** Y. Iwasaki, Tokyo Institute of Technology, Dept of Civil Engineering; M. Kamo, Advanced Industrial Science and Technology, Research Institute of Science for Safety and Sustainability. Estimating the "safe" concentration (e.g., predicted no effect concentration), below which unacceptable effects will not occur, is essential in ecological risk assessment (ERA) of chemicals. ERAs have been generally performed based on the individual-level effects derived from

laboratory toxicity tests. In this context, the species sensitivity distribution (SSD) approach (Posthuma et al. 2002) is widely used to determine the safe concentration. The SSD is a statistical distribution describing the sensitivity of species to a chemical substance, and its 5th percentile concentration (HC5) has been frequently applied as the safe concentration. The typical goals of ecological risk managements are to protect populations, communities, and ecosystems. Although the SSD approach can be used to predict the effects at such levels, the estimated HC5 is still based solely on the individual-level effects. Therefore, more ecologically-relevant methods, such as population modeling, are required to directly assess the impacts at the population and higher levels. The population-level ERA is often conducted by calculating the threshold concentration of a target chemical, which leads to the zero-net population growth. Using the SSD approach with such threshold concentrations for several biological species, population-level HC5 (PHC5) proposed by Kamo and Naito (2008, Hum Ecol Risk Assess) can be estimated. This approach may provide useful information to determine the safe concentration at the population and higher levels. However, since PHC5 was estimated only for zinc (Kamo and Naito 2008), the utility of the approach is not well examined. In the present study, we estimated the PHC5 for copper using the population-level SSD and examined the utility of this approach by comparing it with the HC5 and field-derived safe concentrations.

**WP210 Evaluating the Relative Contributions of Dietary and Waterborne Lead to Toxicity in Rainbow Trout** D. Alsop, McMaster Univ, Biology; J. Chowdhury, International Lead Zinc Research Organization, Inc.; C. Wood, McMaster Univ, Biology. The toxic interactions between waterborne and dietary metals have received little attention in fish, although aquatic animals commonly face multiple routes of exposure. The purpose of this study is to determine the toxic contributions of waterborne and dietary Pb to juvenile rainbow trout over 7 weeks. For the waterborne portion of the experiment, trout were exposed to control water and six concentration of Pb ranging from 4 to 800 µg/L in moderately hard water. Fish were fed 10% body weight per day with live aquatic worms (*Lumbriculus variegatus*). Mortalities were observed in the highest Pb exposure (800 µg/L) over the course of the experiment. Even so, there were no effects of waterborne Pb on specific growth rate at any concentration of Pb over 7 weeks. With a view to developing dietary Pb-exposure doses for trout, *Lumbriculus* were maintained in control water and exposed to the same six concentrations of waterborne Pb. Metal accumulation in the worms was concentration-dependent and increased linearly without reaching steady-state over 7 weeks. These experiments set the stage for the current work that is directly addressing the interactions of dietary and waterborne metal exposures. Trout will again be exposed to waterborne Pb, and will be fed unexposed or Pb-exposed *Lumbriculus*. Fish in control waters will also be fed control and Pb-exposed worms. Survival, growth, Pb tissue accumulation and a variety of physiological endpoints will be monitored in the fish. These experiments should elucidate the relative toxic contributions of these two routes of metal exposure; the findings will be discussed in the context of environmental water quality criteria (supported by ILZRO).

**WP211 Evaluation of Mn and As Exposure of a Goby Fish (*Gymnogobius isaza*) in the Bottom of Hypoxic Lake – Analytical and Experimental Approaches** T. Itai, D. Hayase, Y. Hyobu, Ehime Univ, Center for Marine Environmental Studies (CMES); S.H. Hirata, Tottori Univ, Dept of Regopnal Environment; M. Kumagai, Lake Biwa Environmental Research Institute; S. Tanabe, Ehime Univ, Center for Marine Environmental Studies. In December 2007, mass mortality of Isaza (*Gymnogobius isaza*), an endemic goby fish in Lake Biwa, Japan, was observed. As dissolved oxygen (DO) level in lake bottom was low (< 1 mg/L) due to incomplete vertical mixing of lake water caused by warm winter, oxygen deficiency might be the most plausible reason for mass mortality. However, we found that average concentrations of Mn and As in dead Isaza during the mortality event (MI) were 96 and 16 times higher than that of live Isaza (LI), respectively. Since Mn and As have toxic properties for organism and can be mobilized from sediment under reducing condition, exposure to these elements in the lake bottom with progressing hypoxia should also be verified. A critical rebuttal to the theory of Mn and As enrichments in MI was the secondary accumulation scenario stating that Mn and As have been accumulated from surrounding media after mortality. We considered three approaches to test the secondary accumulation scenario: (i) organ distribution analysis, (ii) speciation approach using X-ray absorption fine structure (XANES), (iii) absorption test for dead

Isaza. If significant secondary accumulation occurred, concentration of As and Mn should be higher in the surface tissues such as skin and gill rather than internal organs. In our organ distribution analysis, however, higher level of Mn and As in MI than LI was not only observed at skin and gill, but also at viscera. Speciation of Mn and As measured by XANES indicated that predominant Mn species were Mn<sup>2+</sup> ion, whereas high proportion As were associated with S such as As-glutathion. Since As in the lake bottom water and sediment were mostly arsenate, presence of As-S bonding from internal tissues suggested possible transformation of As after exposure. Finally, the results of absorption test showed that Mn levels in the organs increased depending on the Mn concentration in test water, whereas As levels in the organs did not increase even if the arsenate or arsenite levels in test water were 10 times higher than the naturally observed level. These results suggest that secondary accumulation of Mn cannot be ruled out, whereas high As levels in mortality Isaza reflects possible exposure before mortality. The results of this study suggest that hypoxia-induced mobilization of trace metals and its effect on aquatic organisms is an important issue in environmental toxicology.

**WP213 Influence of Humic Acid on Ag Uptake and Toxicity to Green Algae** Z. Chen, N. Paquet, C. Porcher, P.G. Campbell, INRS – Eau, Terre et Environnement; C. Fortin, INRS – Eau, Terre et Environnement, Université du Québec. Over the years, the biogeochemical cycle of silver has been disturbed by different anthropogenic activities, from photographic processes to nanoparticles. To better understand the relationship between silver speciation and bioavailability in natural freshwaters, short-term and long-term silver uptake and toxicity experiments were carried out with two species of green algae (*C. reinhardtii* and *P. subcapitata*) in the laboratory over a range of silver concentrations (1 to 200 nM) and in the presence or absence of well characterized natural dissolved organic matter (Suwannee River Humic Acid). The presence of DOM added a layer of complexity since Ag-DOM interactions cannot be predicted accurately with thermodynamic models. We thus determined the free Ag<sup>+</sup> concentration in the exposure solutions using an equilibrium ion exchange technique (Fortin & Campbell 1998 Int J Environ Anal Chem 72:173-194). The Biotic Ligand Model (BLM) predicts that for a given free metal ion concentration metal uptake should remain the same, in the presence or absence of a metal-complexing ligand such as DOM. However, short-term Ag uptake in the presence of DOM was greater than would be anticipated on the basis of the measured free Ag<sup>+</sup> concentration. For the long-term toxicity experiments with *P. subcapitata*, the intracellular concentration of silver required to inhibit growth by 50% (EC50 based on Ag cell quota at 48 and 72 h) was lower in the presence of DOM than in its absence; in other words, internal Ag is more toxic in the presence of DOM. Possible explanations for these results are discussed, including kinetic limitations on silver uptake and direct effects of DOM on the test algae.

**WP214 Influences of Salinity on Acute Copper Toxicity in Blue Crab, *Callinectes sapidus*** K. Chan, Univ of Miami, Rosenstiel School of Marine and Atmospheric Science; M. Grosell, Univ of Miami, RSMAS. Copper targets mainly the osmoregulatory processes and sensitivity to copper is influenced by ambient salinity. Osmoregulating organisms, like the killifish, *Fundulus heteroclitus* has been demonstrated to be least sensitive to acute copper toxicity when exposed in salinities iso-osmotic to their extracellular fluids. For this fish, sodium gradients across the gill epithelium are the key parameter that determines the sensitivity to acute copper exposure across salinities. However, there is limited information regarding acute copper toxicity in euryhaline invertebrates such as crustacean at different salinity. The blue crab, *Callinectes sapidus*, like *F. heteroclitus* is a strong osmoregulator, yet display different osmoregulatory strategies by being isoosmotic in seawater and hyperosmotic at intermediate and low salinities. Thus, the prediction currently being tested is that blue crabs will be least sensitive in seawater and show a gradual increase in sensitivity with decreasing salinity. *C. sapidus* are acclimated to different salinities and subjected to standard 96h LC50 copper tests. Result from blue crabs to date indicate little, if any, need to transport sodium at higher salinities (20-30 ppt) but copper exposure at high concentrations nevertheless cause toxicity. At high salinities a dose dependent increase of hemolymph total CO<sub>2</sub> was observed following 96h of exposure indicating a metabolically compensated respiratory acidosis. Sensitivity of blue crabs at lower salinity as well as mechanisms of toxicity is currently being tested.



**WP215 Interactions of Waterborne Cadmium and Dietary Calcium on Fitness and Mitochondria Bioenergetics in Vivo in Rainbow Trout, *Oncorhynchus mykiss*** R.C. Adiele, Univ of Prince Edward Island, Dept of Biomedical Sciences, Atlantic Veterinary College, Univ of Prince Edward Island, Graduate Student - Toxicology; C. Kamunde, Univ of Prince Edward Island, Associate Professor, Univ of Prince Edward Island, Assistant Professor. Rainbow trout were exposed to sublethal waterborne Cd (5 and 10 µg/l) and dietary Ca (60 mg/g), individually and in combination, for 30 days to elucidate the interactive effects and evaluate the toxicological significance of mitochondrial responses to these cations in vivo. Indices of fish condition and mortality were measured and livers, centers of metabolic homeostasis, were harvested to assess mitochondrial function and cation accumulation. All indices of condition assessed (body weight, hepatosomatic index and condition factor) were reduced in all the treatment groups. Mortality occurred in the Cd-exposed groups with dietary Ca partly protecting against or enhancing it depending on the Cd exposure concentration. State 3 respiration was inhibited by 30, 35 and 40% in livers of fish exposed to Ca, Cd and Cd+Ca, respectively, suggesting reduced ATP turnover and/or impaired substrate oxidation. While the phosphorylation efficiency was unaffected, resting (state 4) and state 4+ (+ oligomycin) respirations were inhibited by all the exposures. Mitochondrial coupling was reduced and transiently restored denoting partially effective compensatory mechanisms to counteract Cd/Ca toxicity. The measured respiratory dysfunction was associated with accumulation of both Cd and Ca in the mitochondria. Although fish that survived acute effects of Cd and Ca exposure apparently made adjustments to energy generation such that liver mitochondria functioned more efficiently albeit at reduced capacity, reduced fitness was persistent possibly due to increased demands for maintenance and defense against toxicity. Overall, interactions between Cd and Ca on mortality, condition indices and mitochondrial responses were competitive or cooperative depending on exposure concentrations.

**WP216 Metal Accumulation and Sublethal Effects in the Sea Anemone, *Aiptasia pallida*, After Waterborne Exposure to Metal Mixtures** G.K. Bielmyer, Valdosta State Univ, Biology, Valdosta State Univ, Dept of Biology; J. Brock, Valdosta State Univ. Metal contamination is common in marine environments, predominantly due to anthropogenic inputs, yet the effects of metals on symbiotic cnidarians are largely understudied. To further understand the impact of elevated metal concentrations on marine symbiotic organisms, a toxicity study was performed using the model sea anemone, *Aiptasia pallida*. *A. pallida* were exposed to a control or a metal mixture (Cu, Zn, Ni, and Cd) at three exposure levels (low, medium, high) for 7 d. Anemones were then transferred to clean seawater for an additional 7 d after the metal exposure to assess depuration and recovery. Metal accumulation, behavior, protein concentration, activity of the oxidative stress enzymes, catalase and glutathione reductase, and activity of the enzyme carbonic anhydrase were measured at 0, 1, 3, 5, 7, and 14 d. Additionally, algal photosynthetic parameters and density of the symbiotic algae, *Symbiodinium* sp., within the anemone were also quantified at the beginning and end of the metal exposure. *A. pallida* rapidly accumulated all of the metals in a time and concentration dependent manner. Enzyme activity followed a similar pattern and varied with exposure concentration. Understanding how this organism deals with toxicity after mixed metal exposure in the laboratory may allow better understanding about how symbiotic cnidarians respond to metal polluted aquatic environments.

**WP217 Metal Partitioning Among Pools of Cytosolic Ligands in Larvae of *Chaoborus punctipennis* Living Along an Environmental Trace-metal Gradient** M. Rosabal, INRS – Centre Eau Terre Environnement, Eau Terre Environnement (INRS-ETE); L. Hare, P. Campbell, INRS – Centre Eau Terre Environnement. Larvae of the dipteran insect *Chaoborus punctipennis* are an effective sentinel for estimating the exposure of lake-dwelling animals to cadmium (Cd) and nickel (Ni), in part because they are very tolerant to these metals. Heat-stable proteins play an important role in the intracellular sequestration and detoxification of metals in these sentinels. However, the distributions of metals among ligand pools within this cytosolic fraction are unknown. Metal-binding proteins are included in this fraction, especially metallothioneins and metallothionein-like proteins which have been associated with enhanced metal tolerance in species living in metal-contaminated environments. We set-out to measure the distributions of Cd, Cu, Ni and Zn among various cytosolic ligand pools in *C. punctipennis*. Larvae of *C. punctipennis* were collected from nine lakes located on the Canadian

Precambrian Shield in the vicinity of metal smelters located in Rouyn-Noranda (Quebec) and Sudbury (Ontario). To evaluate metal partitioning, the cytosol was fractionated by size-exclusion high-performance liquid chromatography to obtain three metal-ligand pools: a high molecular weight pool, “metallothioneins” and a low molecular weight pool. Metals in each pool were measured by ICP-MS. Our results highlight the importance of the “metallothionein” pool in metal sequestration in the intracellular environment of this insect, presumably contributing to the presence of *Chaoborus* larvae in highly metal-contaminated environments

**WP218 Metal Partitioning Among Pools of Cytosolic Ligands in Larvae of the Insect *Chaoborus punctipennis* Living Along an Environmental Trace-metal Gradient** M. Rosabal, L. Hare, P. Campbell, INRS – Centre Eau Terre Environnement, Eau Terre Environnement (INRS-ETE). Larvae of the dipteran insect *Chaoborus punctipennis* are an effective sentinel for estimating the exposure of lake-dwelling animals to cadmium (Cd) and nickel (Ni), in part because they are very tolerant to these metals. Heat-stable proteins play an important role in the intracellular sequestration and detoxification of metals in these sentinels. However, the distributions of metals among ligand pools within this cytosolic fraction are unknown. Metal-binding proteins are included in this fraction, especially metallothioneins and metallothionein-like proteins which have been associated with enhanced metal tolerance in species living in metal-contaminated environments. We set-out to measure the distributions of Cd, Cu, Ni and Zn among various cytosolic ligand pools in *C. punctipennis*. Larvae of *C. punctipennis* were collected from nine lakes located on the Canadian Precambrian Shield in the vicinity of metal smelters located in Rouyn-Noranda (Quebec) and Sudbury (Ontario). To evaluate metal partitioning, the cytosol was fractionated by size-exclusion high-performance liquid chromatography to obtain three metal-ligand pools: a high molecular weight pool, “metallothioneins” and a low molecular weight pool. Metals in each pool were measured by ICP-MS. Our results highlight the importance of the “metallothionein” pool in metal sequestration in the intracellular environment of this insect, presumably contributing to the presence of *Chaoborus* larvae in highly metal-contaminated environments.

**WP219 Pb and Zn Toxicity to Mussel and Echinoderm Larvae: Effects of Salinity and DOC** S. Nadella, McMaster Univ, Biology; S. Smith, Wilfrid Laurier Univ, Chemistry; A. Bianchini, Federal Univ of Rio Grande; M. Tellis, McMaster Univ, Biology; R. Diamond, Wilfrid Laurier Univ, Chemistry; C. Wood, McMaster Univ, Dept of Biology. We investigated the influence of DOC and salinity on the toxicity of Pb and Zn to early life stages of two blue mussel species (*Mytilus galloprovincialis*, *Mytilus trossolus*) and one echinoderm, the sea urchin (*Strongylocentrotus purpuratus*) in 48 or 72 h development tests. In the absence of Pb or Zn, salinity thresholds for normal development were 30 ppt for *S. purpuratus*, 25 ppt for *M. galloprovincialis*, and 20 ppt for *M. trossolus*. For Pb in 100% seawater (33 ppt), the three species exhibited comparable sensitivity. EC50's were 63 (36-94) µg Pb/L for *M. galloprovincialis*, 45 (22-72) µg Pb/L for *M. trossolus*, and 74 (50-101) µg Pb/L for *S. purpuratus* respectively, from dissolved Pb concentrations. Salinity (21 – 33 ppt), evaluated in *M. trossolus*, had no significant effect on Pb toxicity. The addition of DOC from fresh water (Nordic Reservoir) and a marine DOC collected inshore in Brazil decreased the toxicity of Pb moderately in both *M. galloprovincialis* and *M. trossolus*, with EC50 values approximately doubling. However, the effect was not concentration-dependent over the range 2.1-10.5 mg C/L. Low salinity (21 ppt) did not enhance the protective effect of DOC. DOC additions actually increased the toxicity of Pb to *S. purpuratus*. Good correlations were observed between whole body Pb burden and % mortality in both *M. galloprovincialis* and *S. purpuratus* embryos with a 48-h LA50 (BLM parameter) of 575 (251-1000) µg Pb/g in the mussel and a 72-h LA50 of 316 (141-741) µg Pb/g in the sea urchin. For Zn in 100% seawater (33 ppt), the three species again exhibited comparable sensitivities. EC50s were 172 (126-227) µg Zn/L for *M. galloprovincialis*, 135 (103-170) µg Zn/L for *M. trossolus*, and 151 (129-177) µg Zn/L for *S. purpuratus*, from dissolved Zn concentrations. Thus Zn was somewhat less toxic than Pb. The addition of the same two DOCs (2 – 12 mg C/L) had no significant effect on Zn toxicity to blue mussels, but exacerbated Zn toxicity to urchin larvae. As with Pb, good correlations were again observed between whole body Zn burden and % mortality in both *M. galloprovincialis* and *S. purpuratus* embryos with a 48-h LA50 of 759 (617-776) µg Zn/g in the mussel and a 72-h LA50 of 398 (347-575) µg Zn/g in the sea urchin. Thus despite differences in EC50's between Pb and Zn, LA50's were

comparable. These data will be useful in developing BLM's for Pb and Zn in estuarine and marine environments (ILZRO, IZA, NSERC CRD program, IRC program of CIDA/CRC).

**WP220 Quantification of Toxic Metals Carried by Plastic Litter Over a Beach** E. Nakashima, A. Isobe, S. Kako, Ehime Univ; S. Magome, Sanyo Techno Marine, Inc.; N. Deki, Ehime Univ; T. Itai, Ehime Univ, Center for Marine Environmental Studies (CMES); S. Takahashi, Ehime Univ. In East China Sea, an amount of marine litter was found on the beaches, due to rapid economical growth in the neighboring countries. Although this litter poses a great threat to marine wildlife by ingestion of plastics and entanglement in drift nets, few reports on chemical pollution have also been reported. This background prompted us to establish a study to quantify the chemical substances carried by litter in Ookushi beach, Goto Island, Nagasaki, Japan. Firstly, the weight of beach litter was estimated 716±259 kg in Ookushi beach, Goto Island. This was calculated from both the coverage area of beach litter (123.5 m<sup>2</sup>) using balloon camera and the weight measurement of litter in 4 m<sup>2</sup> unit area (5.8 kg/m<sup>2</sup>). Among randomly collected beach litters, Plastics prevail among various materials on the ten boxes of the Ookushi beach, the mass of plastics accounts for 74% (i.e., 530±201 kg), in which five polymers such as PE, PP, PET, PVC and PS accounted for 98%. Secondly, the levels of heavy metals were estimated in litter. Among them the toxic metals, lead (Pb) was the most common toxic metal detected in plastic litter on Ookushi beach. Therefore, we had estimated the total weight (M) of Pb carried by plastic litter by multiplying the estimated mass with the concentration of Pb in each polymer type: On the other hand, Pb contained in plastic PVC fishing floats exceeded 100 mg/kg which is regulated by EU regulation on packaging and packaging waste. This Pb in polymers is used for pigments and might be released into the beach environment when the plastic undergoes degradation. In order to examine Pb leaching from PVC fishing floats, we had conducted several leaching tests. Although the leaching rate (µg/h) of lead from fishing float declined exponentially with time, Pb is leaching from PVC floats. This fact suggested that these Pb may affect the marine environment in the long term.

**WP221 Reducing Metal Mine Effluent Effects on Fathead Minnows (*Pimephales promelas*) Through Water Chemistry Modifications** J.D. Queller, Univ of Saskatchewan, Biology; S. Niyogi, Univ of Saskatchewan, Dept of Biology; M. Dube, Univ of Saskatchewan, School of Environment and Sustainability. Treated metal mine effluents (MMEs) are discharged into waterways and may impact invertebrate and fish populations in those receiving environments. Past studies have shown elevated tissue metal concentrations and altered reproduction in fathead minnows [FHM (*Pimephales promelas*)] exposed to an environmentally relevant concentration of a Canadian nickel mine effluent [45% process water effluent (45% PWE)]. Alteration of the water chemistry parameters, such as increased pH and DOC, is known to reduce the toxic effects of metals under acute exposures. A 21-day multi-trophic exposure was performed using FHMs and *Chironomus dilutus* to determine if these protective effects continue under chronic exposures to a metal mixture (45% PWE). Six treatments were assessed: (1) normal reference water (pH=7.7), (2) reference water with pH increased to ~8.1, (3) reference water with humic acid added, (4) normal 45% PWE (pH=7.2), (5) 45% PWE with pH increased to ~8.1, and (6) 45% PWE with humic acid added. Increasing the pH to 8.1 and increasing humic acid concentrations in both reference water and 45% PWE each resulted in higher cumulative total egg production relative to normal reference and normal 45% PWE exposed FHMs. Normal 45% PWE exposed FHMs showed a decrease in cumulative total egg production relative all other FHMs. However, tissue metal concentrations remained similar between all 45% PWE exposed FHMs regardless of the increase in pH and humic acid. These results suggest that increasing pH and DOC may mitigate some effects of metal mine effluent under chronic conditions. Further work will explore the pathways under which these water chemistry parameters reduce the reproductive effects.

**WP222 Selenium Bioaccumulation in Littoral and Pelagic Food Webs in Lakes Near Metal Smelters** D.E. Ponton, INRS – Centre Eau Terre Environnement, Eau, Terre et Environnement, Institut National de la Recherche Scientifique, ETE, Institut National de la Recherche Scientifique, Eau, Terre et Environnement; L. Hare, INRS-ETE. Aquatic animals are reported to take up selenium (Se) mainly from their diet. Thus changes in diet can alter Se exposure. Within a lake, changes in diet can occur as

animals grow and as they move between the littoral and pelagic zones to feed. Among lakes, food chain structure and Se exposure can be influenced by industrial emissions. All of these factors likely influence Se concentrations in predatory midge larvae (*Chaoborus*) and in yellow perch (*Perca flavescens*), both of which can survive in metal-contaminated lakes. To explain Se concentrations in these predators, we measured Se in various compartments of the pelagic and littoral zones in lakes located at various distances from metal smelters in northern Ontario (Sudbury) and Quebec (Rouyn-Noranda); that is, lake water (selenite, selenate and organic selenium), suspended particulate matter (bacteria and microalgae < 64 µm), zooplankton (prey of *Chaoborus*), littoral invertebrates (prey of yellow perch) and the predators themselves. In all food-chain components, we measured the isotopic signatures of stable nitrogen, so as to estimate trophic position, and carbon and sulphur, so as to determine the source of energy and Se for the food chains. These data allowed us to explain the influence of trophic position and contaminants on Se bioaccumulation by animals in lakes on the Canadian Shield.

**WP223 Sensitivity of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*) to Copper, Cadmium, and Zinc in Acute Exposures** R.D. Calfee, USGS, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; E.E. Little, H.J. Puglis, USGS, Columbia Environmental Research Center; E. Beahan, US Geological Survey. Larval recruitment failure resulting from chemical contamination is thought to be a factor in the endangered status of white sturgeon populations of the Upper Columbia River (UCR). Previously we determined that early life stages of Kootenai and Columbia River sturgeon were sensitive to very low concentrations of copper that may occur as a result of metals extraction and smelting activities in sturgeon habitats. To confirm these observations we conducted 96-hour exposures of white sturgeon from the Upper Columbia River at various stages of development from 2 to 98 days post-hatch to copper, cadmium and zinc under flow-through conditions. We also conducted similar exposures with rainbow trout to determine sensitivity of a commonly tested species. Companion studies evaluated the chronic toxicity of copper, cadmium, zinc and lead to sturgeon and trout and the toxicity of whole-sediments from the UCR to sturgeon. The acute data indicate that sturgeon sensitivity to copper decreases markedly during development with the most sensitive stages occurring between 16 and 30 dph. Sturgeon were less sensitive to cadmium and zinc with LC50 values well above environmental concentrations, however sublethal effects were evident with changes in behavior. Sturgeon were most sensitive to cadmium and zinc at 61 dph. Rainbow trout proved to be less sensitive during the very early life stages (1-46 dph) with LC50 values up to 7 times less toxic for copper, but were more sensitive to cadmium and zinc than white sturgeon. Our results indicate that exposure to metals may be a factor in early life stage survival of white sturgeon.

**WP224 Sensitivity of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*) to Selected Metals in Chronic Water-only Exposures** N. Wang, US Geological Survey, Columbia Environmental Research Center; C.G. Ingersoll, USGS, Columbia Environmental Research Center; W. Brumbaugh, US Geological Survey, Columbia Environmental Research Center; J.L. Kunz, USGS; R. Consbrock, D.K. Hardesty, US Geological Survey. White sturgeon (*Acipenser transmontanus*) in the trans-boundary reach of the upper Columbia River (UCR) in eastern Washington State, USA are experiencing poor recruitment. Limited toxicity data indicated that early life stages of white sturgeon might be more sensitive to copper, cadmium, or zinc than test organisms used to derive the USEPA ambient water quality criteria. However, insufficient information is available to define toxicity thresholds for these metals. The objective of this study was to evaluate the sensitivity of white sturgeon to copper, cadmium, zinc, and lead in chronic water-only exposures conducted for up to about 8 weeks. Rainbow trout (*Oncorhynchus mykiss*) were also conducted under similar test conditions to determine the sensitivity of white sturgeon relative to this commonly tested species. Companion studies evaluated the acute toxicity of copper, cadmium, and zinc to sturgeon and trout and the toxicity of whole-sediments from the UCR to sturgeon. For each of the four tested metals, three chronic toxicity tests were conducted in flow-through diluter systems: (1) Continuous Exposures (about 8 weeks) starting with 1 day post-hatching (dph) fish, (2) Stage 1 Exposures (2 to 3 weeks) with 1 dph fish, and (3) Stage 2 Exposures (4 weeks) with about 28 dph fish. Preliminary results based on survival endpoint indicate that white sturgeon were 8- to 32-fold more sensitive to copper or lead, and were equally or slightly more sensitive



to cadmium or zinc compared to rainbow trout. Larvae of both species in Stage 1 Exposures were equally or more sensitive to the metals than juveniles in Stage 2 Exposures, except that rainbow trout juveniles were  $\geq 4$ -fold more sensitive to cadmium or zinc than larvae. Analyses of growth data (length and weight) are ongoing.

**WP225 Sodium Loss is the Acute Toxic Mechanism of Metals and Other Contaminants in Zebrafish: Implications for Additive Toxicity** D. Alsop, C. Wood, McMaster Univ, Biology. Zebrafish larvae (*Danio rerio*) were used to examine the mechanisms of action and acute toxicities of metals and other contaminants. Larvae had similar physiological responses and sensitivities to waterborne metals as adults. While cadmium and zinc have previously been shown to reduce  $\text{Ca}^{2+}$  uptake, copper and nickel also decreased  $\text{Ca}^{2+}$  uptake, suggesting that the epithelial transport of all these metals is through  $\text{Ca}^{2+}$  pathways. However, exposure to cadmium, copper or nickel for up to 48 h had little or no effect on total whole body  $\text{Ca}^{2+}$  levels, indicating that the reduction of  $\text{Ca}^{2+}$  uptake is not the acute toxic mechanism of these metals. Instead, mortalities associated with metal exposure were effectively related to whole body  $\text{Na}^+$  loss, which decreased up to 39% after 48 h exposures. Additive toxicity was observed between metals. For example, exposure to sublethal levels of nickel (10% of the nickel 96 h  $\text{LC}_{50}$ ) decreased the copper 96 h  $\text{LC}_{50}$  by 58%. In addition, exposure to fluoxetine and other contaminants decreased whole body  $\text{Na}^+$ . Fluoxetine also showed additive toxicity with copper. We have demonstrated that the acute toxicity of a number of contaminants is effectively related to  $\text{Na}^+$  loss, possibly stemming from stress hormone signaling. This common impact on  $\text{Na}^+$  may underlie additive toxicity. Understanding mechanisms will help predict the toxicities of mixtures for water quality criteria, a necessity given that the vast majority of polluted environments are impacted by multiple contaminants (supported by NSERC discovery).

**WP226 Sublethal Effects on Behavior of White Sturgeon (*Acipenser transmontanus*) and Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Copper, Cadmium, and Zinc** E.E. Little, USGS, Columbia Environmental Research Center; R.D. Calfee, USGS, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; H.J. Puglis, USGS, Columbia Environmental Research Center; E. Beahan, US Geological Survey. White sturgeon (*Acipenser transmontanus*), in the trans-boundary reach of the Upper Columbia River have experienced poor recruitment. The potential for contaminant effects on sturgeon spawning behavior and larval mortality is considered a data gap, and raises concerns about additional threats to the rapidly declining populations. Previous studies have shown severe behavioral impairments observed among early life stage white sturgeon during acute (Calfee et al) and chronic (Wang et al) toxicity tests with copper, cadmium and zinc. These behavioral changes occurred rapidly during exposure most often during the first 24 hours, and were similar to those observed when fish were exposed to slag-contaminated sediments from UCR sites (Little et al). Sturgeon behavior was rapidly impaired and to the extent that survival in the field would be jeopardized, as fish would be swept downstream, or readily captured by predators. The objectives of this investigation are to conduct behavioral observations during a series of acute metals exposures to determine time to effect during early life stage development, and to understand the significance of these responses relative to survival of these vulnerable early life stage fish.

**WP227 The Bioavailability and Toxicity of Metals to the Larvae and Juveniles of the Freshwater Unionid Mussel, *Utterbackia imbecillis*** B. Dubansky, Louisiana State Univ, Dept of Biological Sciences, Louisiana State Univ, graduate student; B. Whitaker, Louisiana State Univ, Dept of Biological Sciences; F. Galvez, Louisiana State Univ, Dept of Biological Sciences. Freshwater unionid mussels are the most imperiled group of animals in the world with approximately 70% of known 300 North American species in decline. We are currently investigating the effects of waterborne metal exposures on the larval and juvenile stages of *Utterbackia imbecillis*, and on the mitigating effects of competing and complexing ligands on bioavailability and toxicity metal in this species. We are currently using a novel methodology to infect fish hosts with mussel larvae (glochidia) after exposure of both the host and parental mussel to metals, followed by assessing the efficacy of glochidial attachment and metamorphosis. Additional studies are accessing the metal bioavailability and toxicity in varying water chemistries to juvenile unionid mussels. Our goal is to provide insight into the influence of host physiology on glochidia metamorphic success, and to

develop and utilize this model system to monitor the effects of toxicants on freshwater environments.

**WP228 The Effects of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and Natural Organic Matter Quantity and Quality on Ni Toxicity to *Hyaella azteca*** K. Chan, Wilfrid Laurier Univ; J. McGeer, Wilfrid Laurier Univ, Dept of Biology. The focus of this study is to determine the effects of Ca, Mg and natural organic matter (NOM) on the acute toxicity of waterborne Ni to *Hyaella azteca*. Organisms were cultured in artificial soft water (0.3 mM Ca, 0.075 mM Mg, 0.3 mM Na, 0.3 mM Cl, pH of 7.0 and 20C) and tests followed standard methods. Two sources of amphipods were compared, a commercially available source (Aquatic Research Organisms (ARO)) and organisms collected from Hannah Lake (HL), a lake in Sudbury, ON that was severely impacted by acid and metal inputs from nearby smelters but has since recovered to a significant degree. Lab cultures of HL *Hyaella* were established over 6 months prior to testing and acclimated to very soft water (0.1 mM Ca, 0.025 mM Mg, 0.1 mM Na, 0.1 mM Cl). In addition to survival, the short term uptake of Ni was assessed (whole body dry weight basis) to establish linkages between accumulation and effects in the context of biotic ligand modeling principles. The effects of Ca were tested across a range of concentrations: 0.1, 0.3, 0.75 and 2.0 mM Ca. The  $\text{LC}_{50}$  concentrations of ARO *Hyaella* varied from 1.0 mg/L (95% confidence interval of 0.55 to 1.83) to 3.1 mg/L (95% confidence interval of 2.4 to 4.1), demonstrating protective effects of  $\text{Ca}^{2+}$  competition against  $\text{Ni}^{2+}$ . The HL source of *Hyaella* showed similar protective responses of Ca with Ni  $\text{LC}_{50}$  increasing from 0.56 mg/L to 3.2 mg/L at Ca concentrations of 0.1 mM and 2.0 mM respectively. Increased waterborne Mg at 0.5 mM did not provide protective effects on acute Ni toxicity and appeared to act as a stressor as survival of unexposed controls was reduced. Sources of NOM were collected across a gradient of smelter impacted sites in the Sudbury area and unimpacted reference sites (Muskoka region of Ontario). The protective effects of NOM were assessed on the basis of both quantity (dissolved organic carbon (DOC) concentration) and quality. NOM source quality was assessed biologically (protective effect of DOC on Ni toxicity) as well as spectrophotometrically (absorbance and excitation emission matrix spectroscopy). These results are considered in the context of validating and extending the BLM for Ni and understanding the importance of NOM source differences. This project is part of the TALER (Terrestrial Aquatic Linkages for Ecosystem Recovery) Research Program and is supported by NSERC, NRCan, Vale and Xstrata.

**WP229 The Physiological Implications of pH Manipulation Methods and Their Impact on Observed Toxicity** A. Esbaugh, Univ of Miami, Rosenstiel School of Marine and Atmospheric Science; K.V. Brix, Univ of Miami, RSMAS, Marine Biology and Fisheries; M. Grosell, Univ of Miami, RSMAS. It is well established that environmental pH is an important factor when assessing dissolved metal toxicity in aquatic environments. This is largely due to the effects of pH on metal speciation, which affects the concentration of a toxic metal species relative to the total dissolved metal concentration. To properly evaluate the impacts of pH on metal toxicity, the pH of test media must be experimentally manipulated. Currently three methods are used for such pH manipulation: buffers, carbon dioxide, and acid/base solutions. Although all three methods are effective at altering pH, and consequently metal speciation, they can have dramatically different effects on the physiology of aquatic animals. Since toxicity is the result of cumulative stress owing to metal exposure and other environmental parameters, it seems likely that variable stress stemming from different methods of pH manipulation could impact observed toxicity. The present study employed acute Pb toxicity in the fathead minnow to examine this question, by determining the relative toxicity at pH 7.4 (control), 6.4 and 8.3 using the respective pH manipulation methods. Using the organic buffer MOPS, 96 h  $\text{LC}_{50}$  values were 15% and 29% of the control value at pH 6.7 and 8.3, respectively. In contrast, experiments using HCl or NaOH resulted in  $\text{LC}_{50}$  values that were 42% and 54% of the control value at pH 6.4 and 8.3, respectively. Experiments investigating the effects of carbon dioxide at pH 6.4 will also be performed. Differences in observed acute toxicity between treatments will be discussed with respect to the physiological implications of the various methods.

**WP230 Toxicity of Smelter Slag-contaminated Sediments and Associated Metals from Lake Roosevelt to White Sturgeon** E.E. Little, USGS, Columbia Environmental Research Center; R.D. Calfee, USGS, Columbia Environmental Research Center, US Geological Survey, Columbia



Environmental Research Center. The toxicity of smelter slag-contaminated sediments from the upper Columbia River and metals associated with those slags were evaluated with early life stage white sturgeon (*Acipenser transmontanus*) in 96-hour exposures. Behavioral observations were made to determine if the sturgeon avoided contact with the sediments or avoided dissolved metals. Leachates resulting from holding 75 gms of sediment in 2 liters of water for 96-hours to 2 weeks were nonlethal to sturgeon that were 8 day post hatch (dph), but were toxic to fish that were about 30 dph, suggesting that the latter life stage is highly vulnerable to copper exposure. The toxicity of copper alone was similar to the toxicity of a mixture of copper, cadmium, and zinc. The latter metals were not lethal, but induced adverse behavioral changes including a loss of equilibrium. Fish maintained consistent and prolonged contact with sediments and did not avoid contaminated sediments when provided a choice between contaminated and uncontaminated sediments. Nor did they avoid aqueous mixtures of copper and zinc. The results suggest that metals associated with smelter slags may pose harm to early life stage sturgeon if they occur in areas contaminated by slags and support the need for further investigation of slag toxicity to early life stage white sturgeon.

**WP231 Understanding the Influence of Micronutrients on Cd Uptake and Toxicity in Algae: Refinement of the Biotic Ligand Model** M. Lavoie, INRS-ETE, Biogeochemistry; P.G. Campbell, C. Fortin, INRS-ETE. Within the biotic ligand model (BLM) construct, cations are considered to be simple competitors for metal binding to uptake sites and may offer some protection against metal-induced toxicity. The influence of essential trace metals and cell pre-conditioning to different micronutrient concentrations on metal uptake and toxicity is assumed to be negligible. In order to test this assumption, we monitored Cd uptake and toxicity in a green alga (*Chlamydomonas reinhardtii*) during long-term exposures (60 h) to a range of environmentally realistic free  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  concentrations buffered with NTA. A 200-fold increase of  $[\text{Mn}^{2+}]$  as well as a 100-fold increase of  $[\text{Fe}^{3+}]$  did not affect Cd uptake and toxicity while  $\text{Ca}^{2+}$  effectively offered some protection. Interestingly, we found that a 100-fold increase of  $[\text{Zn}^{2+}]$  and  $[\text{Co}^{2+}]$  decreased Cd toxicity about 3- and 2-fold respectively. Cd uptake and toxicity was not affected by acclimation to the lowest and highest  $[\text{Fe}^{3+}]$ ,  $[\text{Mn}^{2+}]$ ,  $[\text{Co}^{2+}]$  or  $[\text{Zn}^{2+}]$  used. Short-term (30 min) Cd uptake experiments in the presence of varying  $[\text{Cd}^{2+}]$  reveal the presence of two Cd transport sites of different affinities; one of low affinity ( $\text{K}_{\text{Cd}} 1 = 10^{-5.5} \text{ M}$ ) and one of high affinity ( $\text{K}_{\text{Cd}} 2 = 10^{-7.4} \text{ M}$ ). The competitive binding affinity of  $\text{Ca}^{2+}$  to the high affinity  $\text{Cd}^{2+}$  transport sites was only  $10^{-2.4} \text{ M}$  but was much greater for the low affinity Cd binding sites ( $> 10^{-2.4} \text{ M}$ ). Slight non-competitive inhibition of  $\text{Ca}^{2+}$  on  $\text{Cd}^{2+}$  uptake by the low affinity  $\text{Cd}^{2+}$  transport system was also observed. We conclude that low  $[\text{Co}^{2+}]$  and  $[\text{Zn}^{2+}]$  should be taken into consideration as competitors for Cd uptake in *C. reinhardtii*. Furthermore, the protective effect of  $\text{Ca}^{2+}$  on  $\text{Cd}^{2+}$  uptake seems to be more complex than initially anticipated.

**WP232 Site Specific Implications of Mining Regulations: The Case of Zinc in Ross Lake, Manitoba** S. Yacoub, Univ of Toronto, Dept of Chemical Engineering and Applied Chemistry; M. Diamond, N. Gandhi, Univ of Toronto; C. Gueguen, Trent Univ. Ross Lake in Flin Flon, Manitoba, CA has received zinc enriched mine tailing effluents for over 50 years. Due to resuspension of sediments, Ross Lake remains an overall net source of zinc to downstream waterbodies. Recent sampling campaigns, conducted to update fate and speciation models at Ross Lake, have found significant changes in the environmental conditions over the last 10 years. Sediments have gone from anoxic to oxic conditions. pH in the water column has dropped from 8.3 due to the mine operators liming the tailings outflow to ~ 6.5 due to acidifying the outflow to achieve circumneutral pH. This latter change is demanded by recently implemented regulations for the mining sector. The low pH is also due to the breakdown of thiols in the lake where the sulphur originates from the intentional acidification and from the treated tailings effluent. Low pH and dissolved oxygen impact speciation and thus toxicity and bioavailability of metals. In order to assess the overall implications of the regulations we are quantifying the bioavailable fraction of metal using modelling and field in situ testing. The Windermere Humic Aqueous Model (WHAM) was employed to model speciation in Ross Lake, and Diffusion Gradients in Thin Films (DGT) were deployed to measure in situ labile metals concentrations. Field measurements confirm estimates from WHAM of high metal bioavailability under current conditions. This raises questions

about the effectiveness of the application of the regulations at the site specific level.

**WP233 Are Free Ion Activity Models Sufficient Alternatives to Biotic Ligand Models in Evaluating Metal Toxic Impacts in Terrestrial Environments?** M. Owsianiak, Technical Univ of Denmark, Dept of Management Engineering; R. Rosenbaum, Technical Univ of Denmark, Section for Quantitative Sustainability Assessment; H.F. Larsen, M.Z. Hauschild, Technical Univ of Denmark. Metal partitioning between solid and aqueous phases and speciation in soil pore water control the bioavailability of toxic forms of metals, while protons and base cations can mitigate metal ecotoxicity by competitive interactions with biotic ligands. The employment of BLMs to evaluate toxicity potential of metals in soils results in site-specific toxicity scores due to large variability of soil properties and differences in ionic composition. Unfortunately, terrestrial BLMs are available only for few metals and few organisms, thus their applicability to hazard ranking or toxic impact assessment is low and alternatives must be found. In this study, we compared published terrestrial BLMs and their potential alternatives such as free ion activity models (FIAM), for applicability in addressing metal toxic impacts in terrestrial environments. A set of 1300 soils representative for the whole world is employed to calculate EC50 and thereafter hazardous concentration HC50 (geometric mean of all EC50) for these terrestrial organisms, for which both TBLMs and FIAMs are available. Results showed that median HC50 for all soils predicted with BLMs range 2 and 3 orders of magnitude for copper and nickel, respectively. In all cases, predictions of FIAMs fall within the range of values predicted with BLMs, and toxicity ratio of copper to nickel is accurately predicted with both models. Thus, both models are able to distinguish between the two metals in terms of their average toxicity. Given that the calculated toxicity scores show large variability even for soils located in close proximity to each other, selection of FIAMs is also justified in deriving soil quality criteria. It remains to be investigated at what spatial scale the FIAMs are a good alternative to TBLMs in evaluating metal toxic impacts in terrestrial environments.

**WP234 Copper Accumulation and Toxicity in the Lamellae of the Euryhaline Isopod *Excirrolana armata* at Different Salinities** I.F. Barcarolli, Universidade Federal do Rio Grande – FURG; A. Bianchini, Universidade Federal do Rio Grande – FURG, Instituto de Ciências Biológicas. Copper is ubiquitous in aquatic environments, being required for several physiological functions. However, high environmental copper concentrations are proved to be toxic, leading to structural and molecular injuries. In the present study, copper accumulation and effect on sodium regulation and enzyme activities were evaluated in the lamellae of the euryhaline isopod *Excirrolana armata*. Isopods were collected at the Mar Grosso Beach (São José do Norte, RS, Southern Brazil) and acclimated to different salinities (3, 6, 15 and 30 ppt). They were exposed to 0.673, 12.37, 12.42 and 18.94 mg Cu/L at salinities 3, 6, 15 and 30 ppt, respectively. Copper concentrations used corresponded to the 48-h  $\text{LC}_{50}$  values previously determined under the same conditions. After copper exposure, isopods were collected, washed with an EDTA (12 mM) solution to remove the copper loosely bound on the isopod surfaces, and blotted dry in filter paper. Lamellae were then dissected and frozen ( $-80^{\circ}\text{C}$ ) until analysis. An aliquot of samples were dried, digested in concentrated nitric acid, diluted with MilliQ water for copper and sodium concentration measurement (atomic absorption spectrophotometry). Another aliquot of samples was homogenized in a buffer solution and  $\text{Na}^{+}, \text{K}^{+}$ -ATPase and carbonic anhydrase activities were determined (spectrophotometry). Significant lamellar copper accumulation was observed in all salinities tested paralleled with a decrease in the lamellar sodium concentration. A reduced lamellar  $\text{Na}^{+}, \text{K}^{+}$ -ATPase activity was also observed in isopods exposed to copper in salinities 3 and 30 ppt. In turn, carbonic anhydrase activity was inhibited in isopods exposed to copper in salinities 6, 15 and 30 ppt. These findings suggest that copper accumulates in the isopod lamellae and that the mechanism of acute copper toxicity at very low salinity (3 ppt) is associated with an inhibition of the lamellar  $\text{Na}^{+}, \text{K}^{+}$ -ATPase activity paralleled with disturbances in tissue sodium regulation. At higher salinities, this mechanism seems to be associated with an inhibition of the carbonic anhydrase activity, which also leads to disturbances in the lamellar sodium concentration. Data presented are relevant in the scope of the development of a Biotic Ligand Model for brackish and seawater environments (Supported by the International Copper Association, the Brazilian CNPq, and the Canadian International Development Research Centre).

**WP235 The Relationship Between Chemical Extractability and Bioaccessibility of Arsenic in a Former Smelter Site** S. Jeong, Seoul National Univ, Dept of Civil and Environmental Engineering; H. Moon, Seoul National Univ.; K. Nam, Seoul National Univ, Dept of Civil and Environmental Engineering, Seoul National Univ, Dept of Civil and Environmental Engineering. The present study was conducted to determine the bioavailable fraction of arsenic in four different types of soils with organic matter content 1.6 to 4.1% collected from a former smelter site. The main objective was to investigate how the in vitro bioaccessibility could be related to the existing form of arsenic in soil. The five-step extraction method developed by Tessier et al (Analytical Chemistry, 1979, 51(7):844-851) was employed as a means of determining the existing form of arsenic in soil. With the extracted soil samples obtained from each extraction step, in vitro bioaccessibility of arsenic was determined by using the stomach-phase extraction test method developed by the Solubility/Bioavailability Research Consortium (Solubility/Bioavailability Research Consortium, 1999, Appendix C). Total arsenic concentrations in the four soils ranged from 100.1 to 1063.7 mg/kg, and most of them (> 94%) were present as residual form (i.e., arsenic remaining after 4th extraction step), probably due to the long contamination history. The stomach-phase extraction test suggests that the arsenic recovered from 1th to 4th extraction steps, which corresponded to from 0.68 to 7.26% of total concentration, may be bioaccessible. More importantly, some portion of the arsenic which remained in the soil samples after 4th extraction step was also likely to be bioaccessible (i.e., from 0.64 to 11.33% of total concentration). Further analysis indicated that the residual arsenic existed in two forms such as remaining organic matter-bound form and mineral-bound form and the remaining organic matter-bound arsenic probably contributed to the bioaccessible fraction. About 64 – 92% of the arsenic present in the remaining organic matter was bioaccessible whereas only 1.98 – 7.25% was bioaccessible in the mineral-bound form, suggesting that the arsenic associated with organic matter was more bioaccessible. The composition of the residual organic matter (i.e., the organic matter remaining after 4th extraction step) seemed to be also important. A soil sample with 1.6% of organic matter showed higher bioaccessibility compared to another sample with 1.7% of organic matter (i.e., 11.9 and 0.64%, respectively). Humic substance fractionation study revealed that the former had higher humic and fulvic acids content than the latter (i.e., 1,083 and 83 mg/kg, respectively) while the two samples had similar humin contents.

**WP236 Bioavailability of Metals at a Missouri Mine Waste Area** L. Kountzman, Black & Veatch Special Projects Corp, Federal Services Division. The bioavailability of metals in the environment is of great concern particularly near former mine sites, dumps, tailings piles, and impoundments. Soil in these areas may contain higher than average concentrations of many metals (e.g., arsenic, cadmium, chromium, cobalt, copper, lead, nickel, selenium, zinc). In order to evaluate ecological risks associated with elevated concentrations of these metals, the fraction of the total concentration in soil that is bioavailable must be identified. Often, very high percentages (near 100%) of total metals are assumed to be bioavailable. While this assumption is conservative in terms of being protective of human and ecological receptors, it may not be a reasonable estimate of site conditions because the actual bioavailability of metals is often not assessed. The resulting risk may overestimate the true risk of exposure to site media. Overestimation of risk may result in lengthy and costly site remediation that may not be warranted. In order to understand metal bioavailability in soils, chemical and/or biological techniques must be used to measure the portion of metal that is bioavailable to humans and soil organisms. Techniques can range from direct measures of bioavailability such as concentrations in soil organisms to indirect measures such as chemical extractions. For this study, direct measures of metal concentrations were made from samples of plant and earthworm tissue collected from former mine sites, dumps, tailings piles in southeast Missouri. Plant and earthworm tissues were selected because they are important food items for sensitive ecological receptors. Measured results were used to develop site specific uptake factors. Single-variable (e.g., soil concentration) regression models were developed for raw data, as well as ln-ln and log-log transformed data. Multiple regression models including soil pH and fraction organic carbon were also evaluated to determine the effect on model predictions. The best fit estimates (based on the measured concentrations) were compared to default uptake factors used in the development of the EPA Eco Soil Screening Levels (EcoSSLs). Recommendations for incorporating site-specific bioavailability data in ecological risk assessments of mine waste sites are presented and discussed.

**WP237 A Stable Isotope Approach to Investigating Zinc Toxicity and Internal Transport in Barley (*Hordeum vulgare* L.)** J.J. New, J.W. Snodgrass, D. Ownby, Towson Univ, Urban Environmental Biogeochemistry Laboratory; R. Casey, Towson Univ, Urban Environmental Biogeochemistry Laboratory, Towson Univ, Dept of Chemistry; S.M. Lev, Towson Univ, Urban Environmental Biogeochemistry Laboratory. Trace metal in a soil is divided among mineral bound, surface bound and dissolved fractions. These pools can vary significantly among soils and as a result, there may not be a linear relationship between the total metal concentration in a soil and toxicity. Further complicating this relationship is trace metals acting as micro-nutrients (e.g., Zn) for terrestrial organisms. Plants will display a micronutrient exposure response to Zn in the environment. This will not be a linear response to increasing Zn but more of a polynomial response with poor health in Zn limited or excess conditions and good health over a range of optimal Zn. As a result, there is focus on the impact of low soil Zn on crop yields for plants like *Hordeum vulgare* L. (barley) but little attention to the negative impacts at excess Zn, including Zn uptake and storage in soils with elevated Zn. To characterize the Zn effect on barley and the potential for uptake into the roots and shoot, we conducted exposure experiments in OECD soil and concluded Zn within the plant tissue was concentration dependent, with Zn accumulation in the roots and shoots increasing with exposure concentration. Zn uptake in the shoots appears to be well regulated until the exposure concentration approaches the EC<sub>50</sub> of 1500 mg kg<sup>-1</sup> at which point Zn tissue concentrations become correlated with the exposure concentration. Root uptake exhibits a linear response with increasing exposure concentration and is highly correlated with acid extractable Zn in the soil. These results are expected because the roots act as the point of regulation of Zn in *H. vulgare* L. In order to track flow of Zn in *H. vulgare* L. between soil and roots and from roots to shoots, we germinated barley seedlings in a <sup>68</sup>Zn enriched OECD soil for 7 days and transplanted the seedlings into OECD soils with a range of Zn concentrations (100-3750 mg kg<sup>-1</sup>) and a natural isotopic abundance for 7 days. Zn<sup>68</sup> in shoot tissues was rapidly 'lost' as evidenced by the uniformly low fraction of Zn<sup>68</sup> remaining in shoot tissues at all exposure levels. Efflux of <sup>68</sup>Zn to the roots is the most likely pathway. The <sup>68</sup>Zn/<sup>66</sup>Zn ratio in the roots remained elevated and constant despite increasing Zn exposure concentrations. This suggests that the Zn entering the roots must be derived from the shoot, the only <sup>68</sup>Zn enriched source post-transplant. The lack of <sup>68</sup>Zn dilution in the roots is a direct result of the regulation of Zn uptake in the shoots.

**WP238 Assessing Bioavailability of Lead from Soil and Earthworms to Small Mammals Using the Relative Bioaccessibility Leaching Procedure: Study Design** S.R. Beganyi, The Ohio State Univ, Evolution, Ecology, and Organismal Biology; R. Lanno, Ohio State Univ, Dept of Evolution, Ecology, and Organismal Biology. Ecological soil screening levels (SSLs) are based on total lead (Pb) in soil, assuming that 100% of Pb is absorbed by the receptor and reaches target organs. However, this does not give an accurate estimate of Pb risk because not all Pb in the soil is absorbed in the digestive tract and reaches target organs. Bioavailability, or the amount of metal that is absorbed from the digestive tract into the blood stream, is a better estimate of Pb concentrations that may affect receptors. The goal of this study is to assess the bioavailability of lead from soil and earthworms to shrews using the Relative Bioaccessibility Leaching Procedure (RBALP) to determine if bioavailability is less than the assumed 100%. The study site is a private shooting range located in central Ohio. Areas of interest for this study include the main shotfall area that receives spent ammunition from clay pigeon practice, an area where Pb pellets were excavated (Fall 2009), and a reference area that does not receive a significant amount of spent Pb shot. Surface soil from each area will be collected and analyzed for total Pb and extractable Pb. Earthworms will be reared in the three soils for 28 days. After 28 days, soil samples and prey items will be analyzed using the Relative Bioaccessibility Leaching Procedure (RBALP). Lead in tissue samples from field caught mammals will be measured using inductively-coupled plasma mass spectrometry (ICP-MS). Mammal tissue Pb values will be compared to total soil Pb and bioaccessible Pb determined by RBALP to estimate bioavailability. If there is a correlation between field Pb levels in mammals and the relative bioaccessibility, then RBALP may be a useful non-invasive tool for exposure estimates in ecological risk assessment.

**WP239 Elements of Green Energy Technology: Preliminary Hazard Analysis of Rare Earth Metals** D.B. Mayfield, A.S. Lewis, K.R. Reynolds Reid, Gradient. The United States and other western nations are rapidly

expanding mineral resources in order to provide the necessary materials for emerging technologies, particularly green energy technologies. Rare earth elements (lanthanides) are critical to many components (e.g., magnets and batteries) of green energy technologies (e.g., wind turbines, hybrid vehicles). The process of bringing these resources to market (e.g., mining, milling, product development and disposal) may have unintended environmental consequences that may result in exposure to humans and ecological receptors. However, limited studies have been performed to evaluate the potential hazards associated with this group of metals. Further, few regulatory standards are available to manage occupational exposures or environmental releases. Using an existing green chemistry assessment framework, we performed a hazard analysis for rare earth elements. Acute and chronic toxicity studies were also reviewed and summarized to evaluate the potential health effects of lanthanides on humans and the environment. Our preliminary analysis of the more recent studies indicates that rare earth metals demonstrate moderate to elevated toxicity depending on the route of exposure, compound formulation, and/or species tested. These results suggest that further evaluation is warranted due to the growing utility of these elements in technology. Critical data needs, that can provide the basis for guidelines that will be protective of human health and the environment, are also discussed.

**WP240 Hepatic, Renal and Reproductive Effects of Cadmium Contamination in Frog *Microhyla fissipes* Living Downstream of Zinc Mining Area in Thailand** J. Kitana, Chulalongkorn Univ, Dept of Biology; O.

Achayapunwanich, P. Thammachoti, Chulalongkorn Univ, Dept of Biology; M. Othman, Universiti Kebangsaan Malaysia, Environmental Health Program; W. Khonsue, N. Kitana, Chulalongkorn Univ, Dept of Biology. Cadmium is a toxic heavy metal contaminated in environment and caused adverse effects in animal. In Mae Sot District of Tak Province, Thailand, concerns have been raised over the cadmium contamination, potentially due to zinc mining activities. Although there is no report of acute toxicity on animals in this area, the impact of long-term environmental exposure to cadmium on their health are of attention. Water and sediment collected from two potentially cadmium contaminated field sites (low-Cd site and high-Cd site) in Mae Sot during 2008 were analyzed by graphite furnace atomic absorption spectrometry. Year round cadmium contamination in water ranged from 0.0015-0.002 mg/L in low-Cd site to 0.0019-0.0023 mg/L in high-Cd site, while higher levels were found in sediment ranged from 0.1013-0.2206 mg/kg in low-Cd site to 2.9260-3.2888 mg/kg in high-Cd site. *Microhyla fissipes* (n = 20) was collected from each site in 2-month interval during wet season. Detectable level of cadmium residue was found only in the frog collected from high-Cd site. Gravimetric analysis showed that hepatosomatic indices were significantly lower in low-Cd site compared to those of the high-Cd site. Histological analysis showed similar alterations in liver of the frog from both sites including lipid accumulation, necrosis, and fibrosis of the liver tissues. Distinctive melanomacrophage aggregations were found in frog's liver from both sites. Although the number of cells counted was higher in high-Cd site (672.53 cells/mm<sup>2</sup>), the difference was not significant compared to those of the low-Cd site (381.87 cells/mm<sup>2</sup>). Renosomatic indices as well as kidney histology were not significantly different between two sites. Degeneration and pyknosis of renal tubular cells were observed in some area of the kidney tissues. These evidences indicate that environmental cadmium contamination in Mae Sot, even at low level, may cause hepatotoxic and renotoxic effects on the frog living in this area. Reproductive health in term of gonadosomatic indices were significantly different between two sites with the lower GSI in both male and female found in frogs from the high-Cd site. Since exposure to low levels of an endocrine disruptor such as cadmium may induce changes in gonadal development and differentiation resulting in adverse effects on reproduction. Gonadal development will be histologically examined for the assessment of reproductive effects of low level cadmium contamination on this frog species.

**WP241 Advantages to Using Site-specific KOC Values When Assessing Ecological Risk at MGP Sites** R. Devries, T. Rodolakis, AMEC. USEPA

guidance advocates organic carbon partition coefficients (KOC), in concert with Equilibrium Partitioning (EqP) theory, to develop ecological screening benchmarks for organic compounds in sediment (USEPA, 2003). As part of the site investigation and ecological risk assessment of a former MGP site in Massachusetts, AMEC derived site-specific KOCs for polycyclic aromatic hydrocarbons (PAHs) using advanced analytical techniques. Following the USEPA guidance, ΣESBTUs were calculated first using EPA default KOCs and then using site-specific KOCs. Results from the two approaches were then

compared to each other. The partitioning of PAHs from riverbed sediment to porewater using site-specific KOC was a factor of 12 (for alkylated PAH) to 38 (for parent PAH) lower than what was predicted by default KOC. In the context of this MGP site, use of site-specific KOC amounted to a difference of approximately 100,000 sq ft of river bottom that were screened out of the risk assessment with respect to the ESBTU measurement endpoint. This comparison shows that relying on default Koc values may lead to risk estimates that are biased high.

**WP243 Application and Critical Analysis of Brazilian Dredging Regulations** M.C. Lamparelli, CETESB – Companhia Ambiental do

Estado de São Paulo, Hidrobiologia Laboratory; J.E. Bevilacqua, CETESB – Companhia Ambiental do Estado de São Paulo. The application of the 2004 Brazilian regulation, which established an Action List with a lower and upper chemical Action Level for the characterization and destination of dredged material to aquatic environments, and the S.Paulo State regulation for destination on land, resulted in suggestions for their improvement. Although the regulations were not established on simple pass/fail Action Levels, allowing the use of a weight-of-evidence approach, the lack of professional expertise of the stakeholders involved, impaired the integration of all measured physical, chemical and biological characteristics into the final decision. Few alternatives for dredged material other than open water disposal are considered in Brazilian harbors, except for few cases where the material was confined, and practically no beneficial use or treatment exist. Data on sediment quality, available for harbors in several States, and the experience as regulators indicated the necessity to make more explicit the use of bioassays in whole sediments and the adjustment of Action Level 1 to sediment background concentrations of arsenic and nickel on the southeastern coast. It was also verified that the guidelines values adopted, mainly based on ecotoxicological data sets from North American, couldn't be considered restrictive, since values superior to them were rare. Dredging operations in Santos harbor, the largest one in South America, have produced enough knowledge as to establish the minimum requirements for its monitoring. The relationship between quality and quantity of dredged materials at the disposal site, located at 6 miles from the coast, showed that the current direction distributed particles for an area of 7 miles around the site, with C/N ratio indicating a significant continental influence; sublethal effect for organisms and alteration of the benthic community were also verified. Modeling of dredged material transport, indicated that it didn't reach the beaches, as in previous dumping sites and the dispersion allowed the recovery of the area when left undisturbed. Quality assurance of analytical data and information integration can result in more efficient management and monitoring programs. Regulatory framework should clarify aspects related to the diagnosis of the dredged material and include the establishment of a dredging and monitoring plans of the dredged and disposal areas considering potential effects on human health and aquatic environment.

**WP244 Arriving at a Long-term Monitoring Solution for Sediment** A.H. Verwiel, M. Hillyard, E. Conti, AMEC Geomatrix, Inc. In many cases,

environmentally persistent chemicals in sediment have resulted from releases that occurred decades prior, and over the ensuing time the aquatic environment has adjusted and benthic species and aquatic vegetation have returned, making sediment removal damaging to the present ecosystem. While the persistent chemicals remain, they may be inaccessible to biota, which allows for regeneration of the ecosystem. This is particularly true in depositional environments where over time the affected sediments are buried below the biologically active zone, significantly reducing potential ecological effects of the chemicals. If potential ecological risks are identified, the environmental and economic costs/benefits of proposed solutions should be evaluated. A long-term management solution may be selected as the best alternative when the potential damage to the environment by sediment removal is greater than the benefit. However, long-term management solutions are only viable if an effective plan is developed for periodic long-term monitoring, which may last for years or decades. The specific case study is at a former sawmill from which wood-surface-protection chemicals that contained dioxins/furans were released to a tidal slough connected to a large bay. Concentrations of dioxins/furans in the slough (particularly in deeper sediment) were higher than ambient concentrations in the bay sediment. Although shallow sediment concentrations did not present an ecological risk, concentrations in the deeper sediment could present a risk to ecological receptors if the deeper sediment became bioaccessible. Costs associated with focused removal in the areas of highest concentrations at depth were qualitatively



compared to the benefits. Critical to the evaluation was an understanding of the slough hydrology and the potential for sediment deposition and erosion. The cost/benefit evaluation resulted in selection of a long-term monitoring solution. The program includes annual sediment elevation monitoring as well as specific monitoring triggers for significant natural events, including earthquakes, floods, 10-year storm events, and tsunamis, and anthropogenic events, such as changes in land use. The monitoring program has been in place for almost four years, during which time earthquakes, flood events, and tsunamis have occurred, but sediment at depth in the slough has not become bioaccessible.

**WP245 Concentrations and Distribution of PAHs in Sediments from Yatsushiro Sea, Western Japan, and Assessment of the Effects on Benthic Organisms** K. Uehara, Y. GOTO, Kumamoto Univ; S. Shimasaki, Center for Marine Environment Studies, Kumamoto Univ; H. Nakata, Kumamoto Univ, Graduate School of Science and Technology, Kumamoto Univ, Dept of Environmental Science. Recent studies have reported the occurrence of serious contamination by polycyclic aromatic hydrocarbons (PAHs) in the Yatsushiro Sea, western Japan. High concentrations of PAHs were detected in sediments from Tanoura bay, middle-eastern Yatsushiro Sea, at the levels of several tens mg/kg dry wt. basis. However, the number of samples analyzed in the previous study was only five, and little information is available on the comprehensive distribution of PAHs contamination in this bay. In this study we analyzed PAHs in surface sediments collected from 42 stations at inner, middle and outer Tanoura bay. PAHs were detected in all sediments analyzed in this study. The concentrations of PAHs in sediments ranged from 520 to 32,700 ng/g dry wt., at the average value of 5,240 ng/g. High concentrations of PAHs were found in sediments from two stations of southern inner bay (32,700 ng/g and 27,700 ng/g), which were approximately two orders of magnitude greater than those in sediments from reference sites. High molecular PAHs (HM-PAHs), benzo[*b*]fluoranthene, benzo[*a*]pyrene, and indeno[1,2,3-*cd*]pyrene were predominant components in sediments, suggesting the occurrence of specific sources of PAHs in this bay. HM-PAHs concentrations in sediments decreased at the stations of outer bay, implying less mobilization of these compounds in this bay. PAHs concentrations in sediments were compared with the Sediments Quality Guidelines (SQGs), which established by NOAA, in order to evaluate potential effects of PAHs on benthic organisms. Concentrations of acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene benzo[*a*]anthracene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene in sediments exceeded ERL (Effect Range Low) in 28 stations. PAHs levels in sediments from two stations were found to be greater than those of ERM (Effect Range Medium). These results imply that benthic organisms in Tanoura bay may have serious risks by chronic exposure to PAHs, and further studies are needed to evaluate biological effects in this bay.

**WP246 Organochlorine Pesticides and Hydrocarbons in Sediments from the Campeche Coastal Zone, Mexico** L. Alpuche Gual, Campeche Autonomous Univ/EPOMEX Center, Centro Epomex; B. Ramirez-Vargas, E. Cach-Perez, Campeche Autonomous Univ/EPOMEX Center. Coastal zone in Campeche is important for fisheries and touristic activities, and thus for the local economy. This system receives a continuous contribution of toxic compounds from domestic sewage and some industrial activities carried out mainly in the south part of the coastal area. In addition, in past years organochlorine pesticides were sprayed near to the study site for malaria control, and also close to the study site is the Sonda of Campeche, the main oil crude extraction area for Mexico. Overall goal of this study was to evaluate the levels of organic persistent pollutants in sediments (organochlorine pesticides and hydrocarbons). Sediment samples were collected with a stainless steel grab sampler in 15 stations along Campeche, Carmen and Champoton cities. Statistical analysis indicated no significant differences between samples of the three sites studied (Campeche, Champoton and Carmen), although the highest concentrations of hydrocarbons were detected in Carmen, which is a town near the oil platforms. We conclude that the coast zone of Campeche has had impacts of persistent pollutants that are still present together with the addition of hydrocarbons generated by oil activities and waste water residuals of cities.

**WP247 Phosphate Source-sink Dynamics in Androscoggin River** A. Cardamone, Bowdoin College; D. Vasudevan, Bowdoin College, Dept of Chemistry. The Androscoggin River has historically had varying levels of inorganic phosphate (o-P) input from many sources, including pulp and

paper mills, agricultural runoff, and wastewater treatment plants. Since the passage of the Clean Water Act, water quality has greatly improved. As the river becomes cleaner, however, questions remain over whether the o-P currently bound to the sediment will reenter the water and adversely affect the ecosystem. To examine this dynamic, several sediment samples were collected at two locations along the river: Gulf Island Pond (GIP) and Merrymeeting Bay (MMB). Sediments from each site were used in sorption experiments from which the equilibrium phosphorus concentration (EPC) was extrapolated. The EPC value has been extensively used to establish aqueous phosphorus concentrations at which there is no net phosphorus release or uptake by the sediment. At both GIP and MMB, the EPC values indicate an equilibrium between sediment bound phosphate and current pore water and surface water phosphate concentrations. This implies that as phosphate inputs into the river continue to decrease to concentrations less than 0.5  $\mu\text{mol P/L}$  (15 ppb), sediment bound phosphate could be released into the Androscoggin and potentially become a significant new source of phosphate pollution. Furthermore, GIP sediments had higher o-P sorption capacities, surface areas, and extractable iron (ex-Fe) and aluminum (ex-Al) content compared to MMB sediments, indicating the capacity to act as an o-P sink is likely determined by surface area, ex-Fe, and ex-Al content.

**WP248 Potential Effects of PCDD/F, PBDE, Heavy Metals in Sediment Based Laboratory Toxicity Test with Midge and Amphipod; Case Study in Nakdong River, Korea** H. Kim, Pusan National Univ, Dept of Civil and Environmental Engineering; U. Kim, Pusan National Univ; N. Kemble, C. Ingersoll, USGS CERC; J. Oh, Pusan National Univ. The objective of this study is to evaluate relationships between chemically analyzed level of hazardous toxic POPs compounds (e.g., PCDD/Fs, PBDEs) and heavy metals (e.g., 20 regulated heavy metals in Korea compromising Arsenic, Chromium, Copper, Lead, Cadmium and Mercury) and their toxicity results observed from in vitro test in Nakdong river, one of major river basins in Korea, as a case study. Four major river restoration projects were started in 2009 to control flooding, improve water quality, and to restore ecosystem function, the reuse and potential toxicity of dredged sediment are an emerging issue. Sediments from six sampling sites from Nakdong river, which were suspected as contaminated areas, were selected for in vitro toxicity test and chemical analysis. The compositions of sediment samples were 39% sand, 36% silt and 30% clay in average with 35% moisture content. The toxicity tests with the amphipod *Hyalella azteca* (28-d exposures) and midge *Chironomus dilutus* (10-d exposures) were conducted and the toxicity of sediment samples were estimated based on effects on survival, growth, or biomass of test organisms following ASTM and USEPA methods. For the analysis of PBDEs, PCDD/Fs and heavy metals, modified EPA methods were used (1613, 3051). The concentration levels of PCDD/Fs in sediment ranged from 0.055 to 1.497 ng-TEQ/kg-dry and those of PBDEs were from 0.192 to 43.69 ng/kg-dry. The concentrations of most heavy metals were similar regardless of sampling sites and only Chromium and Arsenic exceeded the regulation levels of soil in Korea at all 6 points. The observed PCDD/F was below the interim sediment quality guidelines of USA (2.5 ng-TEQ/kg) but still quite high. Two points near water gate of the river (S3 and S6) was shown the significant toxicity result with *H. azteca* but the level of organic contaminants was high in order of  $S1 > S6 > S4$  for PBDEs,  $S6 > S3 > S1$  in case of PCDD/Fs. Additional evaluations of relationships sediment chemistry and toxicity results will be presented at the conference.

**WP249 Stability and Transport of Formulated NPs Through Environmentally Relevant Porous Media** M.A. Chappell, US Army Corps of Engineers, Environmental Laboratory, US Army Corps of Engineers; L.F. Miller, US Army Engineer Research & Development Center, Environmental Laboratory; C.J. Banks, US Army Engineer Research and Development Center, CEERD-EP-R, US Army Engineer Research and Development Center, Research Biologist; M. Middleton, Badger Technical Services; C. Price, US Army Corps of Engineers. The power of nanotechnology arises from the remarkable surface energy of particles exhibited at the nanometer size range. Materials that are otherwise relatively benign often exhibit unusual reactive, mechanical, or spectroscopic activity. However, there is a great energetic cost associated with retaining particles at these small sizes, and thus, the need for special preparations to create stable nanoparticle (NP) dispersions, such as through severe ultrasonic agitation or chemical modification via application of surface coatings. While the latter preparation provides what are deemed as "thermodynamically" stable dispersions, the trade-off is a resultant uncertainty whether the particles will exhibit

desirable properties. For this work, we investigated the long-term stability of nano silver (nAg) and carbon nanotube (CNT) suspensions by examining the simultaneous dispersion and dissolution behavior of the particles in the presence of background electrolyte, surfactants, and Aldrich humic acid over a 14-day period. Formulated NP dispersions were leached at steady state through highly porous media (e.g., sand, glass beads). Particle size and dissolved solutes were determined in the effluent and NP distribution in the column by in situ EDX mapping. Formulated NP attachment and straining coefficients were determined through analytical modeling of the transport data using colloid transport algorithms. Based on this work, we present numerical calculations of the potential mobility of these dispersions through profile of differently textured soils.

**WP250 Why Be a Benthos? Application of Basic Benthic Ecology to Basic Benthic Ecological Risk Assessment and NRDA** D.W. Smith, Conestoga Rovers & Associates; S.D. Cooper, Univ of California Santa Barbara, Dept of Ecology, Evolution, and Marine Biology. Benthic ecological risk assessment (ERA) and Natural Resources Damage Assessments (NRDA) generally assume that 1) the benthos dominate aquatic ecosystem structure and function AND 2) all benthic macroinvertebrates occur deep within fine sediments (i.e., they are "deep infauna" eating only buried organic matter and exposed only to pore water). Put simply, it is assumed that significant impacts to deep infauna will produce similar impacts on fish production. In reality, benthic macroinvertebrate communities and their importance to ecosystem functions vary greatly across microhabitats, within and across aquatic systems. Like all organisms, macrobenthos require food, shelter from predators and currents, and habitable physical conditions (e.g., enough dissolved oxygen, not too much ammonia). Microhabitats, which provide more of these basic requirements, will engender more benthos (i.e., higher benthic densities, biomass, and production, with higher relevance to ERAs and NRDAs.) For example, in many aquatic systems (most streams, unproductive lentic systems), food quality and quantity for the benthos are highest at the sediment-water interface. In others, food quality and quantity are highest in the water column (more productive lake systems) or on plants (marshes, weed beds). In most aquatic systems, food quantity and quality decrease with depth into the sediments, and more degradable and nutritious organic matter tends to be eaten or metabolized prior to burial. Hence, the production, biomass, and importance of the epibenthos to energy flow will potentially be higher than those of the deep infauna. Similarly, the carrying capacity of different substratum types (fine vs. coarse sediments) and sediment depths can be evaluated with respect to the other critical requirements for life. Using current theory and empirical data, we will test the following hypothesis. The importance of specific benthic communities (deep infauna, shallow infauna, epifauna, epiphyta) to an ecosystem's structure and function depends on the amount and distribution of benthic food, shelter, and habitat conditions. Thus, knowledge of basic benthic ecology has important implications for ERAs and NRDAs. Many aquatic systems are not dominated by fine sediment habitat or by deep infauna. Fish may rely on many food sources other than the deep infauna. Alternate session would be "Evaluating Human Health and Ecological Risk Assessments and Remediation Decisions: is cure .."

**WP251 Altered Offspring Sex Ratios in Birds Living Along a Mercury Contaminated River** A.J. Boulard, College of William and Mary, Dept of Biology; A.E. White, College of William & Mary, Dept of Biology; K.P. Lonabaugh, W.T. Northam, College of William & Mary, Dept of Biology; D.A. Cristol, College of William & Mary, Professor of Biology, College of William & Mary, Dept of Biology. Virginia's South River was contaminated by a point-source of mercury between 1929 and 1950. Mercury is a persistent toxin that is known to impact the fitness of birds inhabiting aquatic environments. Our study examined the effect of mercury exposure on nestling sex ratios in three avian species inhabiting this site and nearby reference sites. In accordance with sex allocation theory, we hypothesized that females inhabiting contaminated sites would (1) be in poor condition, and (2) would produce more female offspring, as females in poor condition are more likely to be successful in competition for mates than males of poor condition. The highly aquatic belted kingfisher (*Megasceryle alcyon*), with the highest mercury levels (~3.5 ppm wet weight), showed a bias toward female offspring (64.17% female), while no bias was found in the highly terrestrial bluebird (*Sialia sialis*), with lowest blood mercury (~1 ppm). Nestlings of both species can be assigned as male or female based on plumage coloration. A molecular PCR technique was used to determine sex of nestling tree

swallows (*Tachycineta bicolor*), a species intermediate between aquatic and terrestrial environments and with intermediate mercury levels (~2.5 ppm). Preliminary analyses indicate a bias toward female offspring in this species (59.1% female), suggesting that mercury contamination weakens breeding females and causes compensatory adaptive adjustment of offspring sex ratios.

**WP252 Considerations for Assessing the Status of Ecosystem Services Following the 2007 Grass Valley Wildland Fire: Thinking Historically** C. Menzie, Exponent; T. Deardorff, Exponent, Senior Managing Scientist. The Grass Valley fire burned approximately 1,247 acres of land to the north-west of Lake Arrowhead in the San Bernardino National Forest in 2007. Any assessment of resource damages to the forest resource from this fire must include an assessment of pre-existing conditions. When considering the ecological service losses following the Grass Valley Fire, we determined that the services were significantly reduced prior to the fire and that the forest was at a high risk of a fire due to considerable impacts from beetles, disease, and drought. Because of the dynamic nature of forests, these pre-conditions can involve a dynamic or changing baseline that includes periodic fires. For example, immediately before the 2007 fire, the available evidence indicated that the environmental conditions and health of the forest that burned in the Grass Valley fire were substantially reduced prior to that fire and shows that most of the trees that were burned in the fire were either dead or dying prior to the fire. Thus, environmental services associated with a healthy and alive forest were also diminished. Following a wildland fire, the habitat changes deemed of value by an analysis may or may not be beneficial to all wildlife. Many of the post-fire changes are necessary for the health of a dynamic ecosystem. The values of forest services are not constant through time and the recovery process is arbitrary and contingencies, including secondary disturbances, impact the process. Distressed ecosystems do not naturally or automatically revert to the conditions observed pre-disturbance, and the extent and rate of recovery of the landscape is far too complex to put a successional and lengthy timeline on it. The integrated services supported by a natural forest are dependent on the presence of a patchwork of tree species, age/size classes, and plant communities in various successional stages. The dynamic and changing nature of forests is what provides for a healthy and functional forest condition and that the ongoing spatial and temporal changes within forests reflect the natural state and very likely the desired state with respect to net environmental services over time.

**WP253 Examination of Mandibular and Maxillary Squamous Cell Proliferation in Mink (*Mustela vison*)** R. Ellick, Michigan State Univ, Animal Science; S. Fitzgerald, Michigan State Univ, Diagnostic Center for Population and Animal Health; J. Newsted, Cardno Entrix; N.M. Bello, Kansas State Univ; J. Link, S. Bursian, Michigan State Univ, Animal Science. Mink (*Mustela vison*) exposed to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and other TCDD-like compounds, such as 3,3',4,4',5-pentachlorobiphenyl (PCB 126), develop an invasive jaw lesion characterized histologically as mandibular and maxillary squamous cell proliferation. Nests and cords of squamous epithelial cells invade the adjacent alveolar bone to cause osteoporosis. Macroscopically, the lesion manifests as swollen and bleeding gums, loose teeth, and eventual tooth loss. In addition to laboratory studies, jaw lesions have been reported in wild mink residing in environments contaminated with TCDD-like chemicals and there is evidence suggesting jaw-related lesions in other wildlife species collected in environments contaminated with TCDD-like chemicals. Commercial blue iris mink have exhibited the gross clinical signs of this lesion without TCDD-like chemical exposure. To date, no studies have been published addressing the etiology of this lesion, but the arylhydrocarbon receptor (AhR) is assumed to be involved because of the lesion's induction by TCDD-like chemicals. To further examine the development of the lesion, two studies were conducted. In the first study, resveratrol, an AhR antagonist, was administered to determine if it could prevent or reduce the development of the lesion. Juvenile mink receiving a single intraperitoneal injection of 30 µg PCB 126/kg bw or a single PCB 126 injection plus 6 daily gavage doses of 50 mg resveratrol/kg bw. Mink were euthanized 9 weeks later, and the jaws were processed for histological assessment. Resveratrol had no apparent effect on the incidence or severity of the PCB 126-induced effects. A second study was conducted to determine if the periodontal disease described in the blue iris strain of mink, which is grossly identical to the PCB 126-induced jaw lesion, was in fact the same lesion. It was shown that the condition in blue iris mink is a destructive inflammatory response rather than an invasive squamous epithelial proliferation. Thus, it is recommended that wildlife species with gross signs

characteristic of the PCB 126 jaw lesion be assessed histologically to rule out other potential causes such as inflammatory disease.

**WP254 Interspecific Effects of Munition Compounds in Three Avian Species: Japanese Quail, Northern Bobwhite, and Zebra Finch** M. Quinn, US Army Public Health Command (prov.), & Preventive Medicine; M. Cook, Oak Ridge Institute of Science and Education; T. Hanna, US Army Public Health Command; A. Shiflett, Oak Ridge Institute of Science and Education; M. Johnson, US Army Public Health Command; K. Gust, E. Perkins, US Army Corps of Engineers. The overall objective of this study was to assess the toxicological and toxicogenomic effects of two munition compounds on three different bird species. This presentation will communicate only the results pertaining to the comparisons of the toxicological effects among the species. Two commonly used model species for avian toxicity tests (northern bobwhite and Japanese quail) and a representative passerine species (zebra finch) were exposed to 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and 4-amino-2,6-dinitrotoluene (4A-DNT) by oral gavage for seven days. Blood was collected following the seventh day of exposure for the following cellularity and chemistry analyses: total erythrocyte and leukocyte counts, hemoglobin, hematocrit, and plasma aspartate aminotransferase, glucose, creatine kinase, triglycerides, uric acid, phosphorus, albumin, globulin, lactate dehydrogenase, and calcium. Of the three species, the northern bobwhite appeared to be most sensitive to 4A-DNT as indicated by mortality and altered levels of red blood cells and plasma proteins, and the zebra finch was most sensitive to RDX as evidenced by mortality and seizures. Although Japanese quail were affected by both chemicals, changes in blood cellularity and chemistry were not as severe and no mortality was experienced due to either of the chemicals.

**WP255 Organochlorine Compounds and Mercury in Bald Eagle Nestlings and Eggs from Coastal Maine** S. Mierzykowski, US Fish & Wildlife Service, Maine Field Office/Ecological Services; L.J. Welch, US Fish and Wildlife Service, Maine Coastal Islands National Wildlife Refuge; C.S. Todd, Maine Dept of Inland Fisheries and Wildlife; B. Connery, National Park Service, Acadia National Park; C. DeSorbo, Biodiversity Research Institute. In 2009 and 2010, bald eagle nestling blood and non-viable eggs were collected along the Maine coast and analyzed for organochlorine compounds and mercury. Nestling plasma (n = 20) contained Total PCB (mean  $54.7 \pm 28.2$  ng/g wet weight, range: 14.6 – 119.1 ng/g) and DDE (mean  $7.4 \pm 5.2$  ng/g, range: 1.8 – 21.2 ng/g) concentrations that were below suggested toxicity threshold levels. Non-viable eagle eggs (n = 9) had substantial amounts of organochlorine compounds (means TCDD-TEQ 251 pg/g, Total PCB 12.76 µg/g, DDE 1.90 µg/g, Total PBDE 765 ng/g, fresh wet weight), but were also below suggested toxicity threshold levels. Mercury in whole blood from eagle nestlings was not elevated (n = 43, mean  $0.194 \pm 0.150$  µg/g wet weight, range: 0.031 – 0.817 µg/g) and below background (< 0.40 µg/g). However, one bird had a mercury blood level in the range classification as moderately elevated (0.40 – 0.69 µg/g) and another had a mercury concentration that would be considered elevated (0.70 – 0.99 µg/g). Mercury in non-viable eggs (n = 9, mean  $0.15 \pm 0.09$  µg/g fresh wet weight) was well below suggested threshold levels for reproductive effects. The results confirm previous research in Maine. Coastal birds appear to exhibit higher organochlorine levels than eagles nesting along rivers and lakes in inland Maine. In contrast, inland birds appear to exhibit higher mercury levels than coastal eagles. Differences in prey bases may be responsible for the variations in contaminant uptake by nest location. In Maine, coastal eagles principally feed on gulls, cormorants, and sea ducks; while inland eagles feed primarily on fish.

**WP256 PCB Concentrations in Tree Swallow (*Tachycineta bicolor*) Eggs Collected from the Upper Hudson River Varies Within a Season and Between Years** K. Dean, Univ of Lethbridge, Neuroscience, Univ of Maryland, post-doctoral research fellow; M. Ottinger, Univ of Maryland, Dept of Animal and Avian Sciences. PCB content of tree swallow eggs collected from two uncontaminated sites (Cobleskill Reservoir, NY and Patuxent Research Refuge, MD) and the Upper Hudson River site in 2006-2008 were compared across years and within the breeding season for each year. Composite samples of two unincubated eggs were separated into chemical free jars and analyzed for PCB congener concentrations. Analyses across all three years showed total PCB concentration, dioxin-like PCB concentration, and non-dioxin-like PCB concentrations were significantly higher (p < 0.05) at the contaminated site. Since tree swallow breeding season begins in

late April and can continue until mid-July, there is sufficient time for female tree swallow PCB body burden to translate into egg deposition. Additionally, it is likely that eggs laid later in the season would contain higher PCB concentrations as females are more likely to be exposed to higher PCB concentrations with increased insect availability during warmer months. When eggs were categorized according to time from initiation of egg laying at the Upper Hudson River, there were increases in PCB concentrations in eggs laid later in the season. These data demonstrate a dynamic uptake of PCBs by females in contaminated regions that reflect their environmental exposure on a seasonal basis, potentially impacting the development of their offspring. Acknowledgements: Supported by the US Fish and Wildlife Service and the Hudson River Natural Resource Trustees. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees or the United States.

**WP257 Recent Experiences in the Conduct of the Passerine Acute Oral Dose Study Required by the USEPA for the Registration of Conventional Pesticide Products** K. Brugger, DuPont Crop Protection; M. Christ, Bayer CropScience; S. Mortensen, BASF. In 2007, Part 158 of Title 40 in the Code of Federal Regulations for the registration and reregistration of conventional pesticide products was revised to add a passerine oral dose (LD50) study to data submissions. The avian oral acute USEPA guideline (OPPTS 850.2100-draft) includes methodology for conducting studies with mallard and bobwhite quail but not song birds. Progress has been made in the last 2 years with method development in the following areas: protocol design, species selection (with a goal to develop this new database of chemical toxicity with a common set of species), bird acquisition (commercially reared vs. wild caught), gender verification, fasting period, dose administration, dose regurgitation, handling stress, determination of general husbandry requirements for each species and data interpretation. Commercially available canaries (*Serinus canaria*) and zebra finches (*Taeniopygia guttata*) proved to be suitable species that can be ordered in sufficient numbers to run limit tests (~20 birds) or full dose response tests (60+ birds). Small tissue or blood samples may be used for rapid and cost-effective DNA verification of gender to ensure equal allocation of individuals among treatment groups. Methods were developed to accurately dose very small birds and selectively replace birds if there is regurgitation. Studies conducted to date indicate that sensitivity of passerines to an active ingredient is generally not greater than that of mallard or bobwhite with most endpoints falling within the same hazard category of previously tested birds and a few endpoints shifting from one hazard category to the next (e.g., practically non toxic to slightly toxic). Regulatory impacts of these studies will be reviewed in light of USEPA OPP methods for risk assessments.

**WP258 Results of Larval Honey Bee Inter-laboratory Validation Tests for Development of a Standardized Test Procedure** M. Patnaude, Smithers Viscient, Ecotoxicology; Z. Huang, Michigan State Univ; J. Hoberg, Smithers Viscient. With no standardized test to assess sensitivity of honey bees to transgenic crops, the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) recruited laboratories to participate in ring testing for this purpose. The objective was to establish a standardized GLP test. Zachary Huang (Michigan State Univ) modified an assay that other scientists developed which serves as the base method. Although originally developed for transgenic proteins, other compounds can be tested under the same design. Presented here are data from the exposures performed at Smithers Viscient, Wareham, MA. The data suggests that this assay can generate consistent results and the larval honey bee assay can provide valuable data for the assessment of honey bee sensitivity to chemicals.

**WP259 Safety Profile of Furfural for Avian Exposure** N. Cowen, toXcel LLC, Associate Scientist; G. Burger, J. Hensley, Agriguard Company, LLC; A. Katz, toXcel, LLC. Furfural (CAS Reg. No. 98-01-1) is registered in the US to control root infesting parasitic nematodes and to reduce fungal plant diseases such as species of *Pythium*, *Phytophthora*, *Fusarium* and *Rhizoctonia*. It acts on contact as an effective liquid crop protection agent and a substitute for soil fumigants, including methyl bromide. Furfural occurs naturally in a wide range of fruits and vegetables, as well as beverages (wine, whiskey), bread and in several essential oils of plants. It is Generally Recognized as Safe (GRAS) for use as a food flavoring agent. Furfural degrades rapidly in soil, air, water and plant surfaces following application to bare ground or foliage. Ecological characteristics of furfural such as avian



toxicity compare favorably with other nematocidal active ingredients, and range from practically non-toxic to moderately toxic. This presentation specifically explores the avian oral, dietary and inhalation routes of exposure for furfural, and gives further explanation to the results of recent studies and their relevance to actual field application exposure.

**WP260 To Depurate or Not to Depurate? Using Earthworm Tissue to Estimate Doses in Ecological Risk Assessment** K.B. Leigh, ENVIRON International Corporation, ENVIRON; M.H. Henning, A.L. Fogg, P. Fuchsman, E. Perruchon, ENVIRON International Corporation; N.E. Dyck, Ontario Ministry of the Environment. In ecological risk assessment, earthworms are often chosen for analysis to represent the soil-dwelling invertebrate community. Earthworms are intimately connected to the soil-by living in constant and direct contact with the soil, as well as regularly ingesting large amounts of soil. They are less mobile than other terrestrial invertebrates, allowing for a more localized assessment, and they have been shown to accumulate soil-associated chemicals into their tissue. For these reasons, earthworms often represent the highest exposure potential from chemicals in soil from a localized area. The collection of earthworm tissue samples allows for the direct measurement of chemicals in the body, thus eliminating the need to use overly conservative literature-based bioaccumulation factors. These body burden concentrations can be used to estimate a daily dose for higher trophic level species, such as the short-tailed shrew (*Blarina brevicauda*). It is important to determine whether or not to depurate (i.e., to allow the earthworms to eliminate their gut contents) prior to laboratory analysis and to account for this factor when estimating higher trophic level doses. Since earthworms' guts typically contain 20 to 30 percent soil, care must be taken to avoid significant overestimation of the total dose due to double-counting of the soil material contained within the earthworms. For this evaluation, earthworms were manually collected from each soil sample location and separated into two groups (one depurated and one non-depurated). Each earthworm group was weighed and analyzed for metals. Using both site-specific and literature information, the relative merits and cost-effectiveness of the depuration process was evaluated. The decision to depurate is predicated on many factors, including cost-effectiveness.

**WP261 A Multigenerational Study Investigating Effects of Dietary Exposure to 17 $\beta$  trenbolone on the Expression of Steroid-responsive Genes in Japanese Quail** Y. Chen, Virginia Tech, Dept of Fisheries and Wildlife Sciences, USGS Patuxent Wildlife Research Center, USGS Patuxent Wildlife Research Center, Patuxent Wildlife Research Center; N. Karouna-Renier, USGS Patuxent Wildlife Research Center, Beltsville Lab ; P.F. Henry, USGS Patuxent Wildlife Research Center, Patuxent Wildlife Research Center; C. Maddox, D. Sprague, Patuxent Wildlife Research Center, USGS, Beltsville Lab. The current study investigated the effects on gonadal function of Japanese quail (*Coturnix japonica*) under continuous exposure to 17 $\beta$  trenbolone, a synthetic anabolic steroid, over three successive generations. Japanese quail were exposed to 5 doses of 17 $\beta$  trenbolone in feed. F0 generation were exposed to treated diet from 6 weeks, F1 generation were exposed in ovo and fed the same treated diet from day 1 of hatch, and F2 exposed solely in ovo as they were provided with untreated feed. F0, F1 and F2 parents were euthanized at 16 weeks of age and expression of sex steroid responsive genes, including Vitellogenin II (VTG), Very low-density apolipoprotein II (apoVLDL II), and ZP1 in liver, and P450 aromatase in the gonads were examined by real-time quantitative PCR. Results across treatment groups within the same generation were compared to evaluate dose-related effects of trenbolone exposure. Results a cross different generations were also compared to evaluate the accumulated, cross-generational effect of long-term exposure to trenbolone. These results, combined with on going analysis on data from endocrine and physiological endpoints collected from the same population, will allow us to assess the cross-generation endocrine disrupting effects of 17 $\beta$  trenbolone in a model avian species.

**WP262 Accumulation and Toxicity of Metals and Organic Compounds to the Oribatid Mite *Oppia nitens*** O.J. Owojori, Univ of Saskatchewan, Dept of Soil Science, Obafemi Awolowo Univ, Zoology; S.D. Siciliano, Univ of Saskatchewan, Dept of Soil Science. The oribatid mite *Oppia nitens* has recently been assessed and proved suitable as a test species for ecotoxicological assessment of contaminated boreal soils. Knowledge of the ecotoxicity of pollutants of different modes of action to this species is necessary for protocol standardization. In this contribution, we assessed the toxicity of four metals (copper, zinc, cadmium and lead) and two organic

chemicals (benzo[a]pyrene and geraniol) to *O. nitens* over a 35-day period. We monitored survival, reproduction and tissue accumulation at the end of the 35 day period. Reproduction was a more sensitive endpoint than survival. The reproduction EC50 values for Cu, Zn, Cd and Pb were 3592, 1997, 145 and 1678 mg kg<sup>-1</sup> respectively while those for benzo[a]pyrene and geraniol were >1600 and 316 mg kg<sup>-1</sup>. The LC50 values for Cu, Zn, Cd and Pb were 3311, 2291, 603 and 6741 mg kg<sup>-1</sup> respectively while those for benzo[a]pyrene and geraniol were >1600 and 251 mg kg<sup>-1</sup>. When using survival data to assess toxicity of metals, the mites showed the highest tolerance to Pb, but with the reproduction data, mites showed the highest tolerance to Cu. This suggests that for a correct evaluation of the toxicity of contaminants to this mite, a battery of endpoints should be used. Although none of the organic chemicals studied were accumulated by the mites, the four metals were accumulated to varying degrees showing the tendency to use body burden of *O. nitens* as an indicator of metal pollution.

**WP263 Assessing the Bioaccumulation and Effects of Select Energetic Compounds on the Earthworm *Eisenia fetida*** R.E. Boyd, Badger Technical Services, LLC, Environmental Laboratory; B.C. Suedel, US Army Engineer Research and Development Center, CEERD-EP-R, Environmental Laboratory, Waterways Experiment Station EP-R; N. Barker, US Army Engineer Research and Development Center; P. Gong, SpecPro Inc., Environmental Services; E.J. Perkins, US Army Engineer Research and Development Center, Environmental Laboratory, Environmental Processes and Effect Division. The use of munitions at military installations has resulted in the inadvertent contamination of abiotic media in these areas. While much experimentation has been performed with energetic compounds associated with these installations, questions remain as to the fate and effects of these materials in the environment and our ability to predict their environmental behavior. Among the energetic compounds most commonly found in soils at contaminated sites are RDX, TNT, and HMX. To improve our understanding of the bioavailability and effects of explosive mixtures, we performed experiments using both field-contaminated and laboratory-spiked soils containing RDX, TNT, and HMX. Field soils containing RDX, TNT, and HMX were collected from a site in the Midwestern and Northeastern US, and an uncontaminated control soil from the Southeastern US was collected for use in the spiking experiments. The experiments were conducted for 4, 14, and 28 days. Spiked soil experiments utilized radiolabeled compounds for more precise quantification. The result of the spiking experiments indicated both chemical bioavailability and physiological effects, such as retarded growth and body fragmentation. Observed physiological effects were similar between spiked soil experiments and field collected soil experiments. In most instances, there was a direct correlation between increased exposure period and soil concentration with increased body burden measurement for both the spiking and field soil experiments. In conclusion, these experiments confirmed physiological effects as well as the extent of bioavailability that some terrestrial invertebrate organisms might exhibit when exposed to the energetic compounds RDX, TNT, and HMX. This research is being used to foster the development of biological tools to help detect and discriminate these energetic compounds at military installations.

**WP265 Characterization of Nanomaterial Test Solutions for Terrestrial Plant Dose-response Studies: A Comparative Study of DLS and SAXS** J. Betts, M. Plocher, Dynamac Corporation; L. Tumburu, National Research Council; M.G. Johnson, USEPA, National Health and Environmental Effects Research Laboratory; G.A. King, Dynamac Corporation; P.T. Rygiel, C.P. Andersen, USEPA, National Health and Environmental Effects Research Laboratory. Industrial applications of nanomaterials have expanded at an increasing rate in recent years, accompanied by the need for comprehensive toxicological assessments to establish environmental health and safety standards. Relatively few studies have examined the effects of nanoparticles on terrestrial plants, and the studies that have been done often provide incomplete characterization of particle solutions, making replication and interpretation difficult. In order to fully assess the potential effects of nanoparticle exposure, it is imperative that solutions are fully characterized and exposure protocols are well documented. Preliminary results from an *Arabidopsis thaliana* Co-O seed germination trial performed with titanium dioxide (TiO<sub>2</sub>) nanoparticles (NP) with five concentrations [0.1-1000 ppm] suggested an apparent retardation in cotyledon expansion at 10 ppm and slowest seed germination at 1000 ppm. It was difficult to interpret these results, however, because despite identical mixing protocols, concentration was not the only factor that differed among our various test solutions. To address

this problem, a comparative study of pre-exposure characterization techniques (Dynamic light scattering/Electrophoretic light scattering [DLS/ELS] & Small-angle X-Ray Scattering [SAXS]) was performed in conjunction with an *A. thaliana* plant exposure study. Nanomaterial properties including mean particle size, particle size distribution (PSD), and suspension stability at various particle abundances were compared. Initial results of TiO<sub>2</sub>-NP suspensions using DLS/ELS suggested an increase in dispersion stability at higher concentrations, which could confound dose-response studies. Such findings highlight the need to consider the constraints associated with characterizing solutions based only on light-scattering measurements. Our results will help to quantify observable differences in physico-chemical characteristics due to variable particle abundance, leading to a better understanding of the limitations of each measurement technique in order to establish more comprehensive and accurate methods for characterizing nanomaterial test solutions for toxicological studies.

**WP266 Characterization of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in White-tailed Deer Along the Tittabawassee and Saginaw Rivers, MI, USA** J. Newsted, R. Holem, D. Kay, Cardno Entrix; A. Blankenship, Univ of Michigan; S. Roark, CH2M; J. Giesy, Univ. of Saskatchewan, Dept Biomedical Sciences and Toxicology Centre. The Tittabawassee River (TR) is located in central Michigan and flows south-east through Midland and into the Saginaw River (SR) and eventually to the Saginaw Bay of Lake Huron. Previous studies have reported elevated soil, sediment, and fish concentrations of dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) downstream of Midland. Given that Michigan has a very active hunting season and concerns have been expressed over the potential risks associated with the consumption of wildlife harvested in the floodplain of both TR and SR. As part of an effort to address these concerns, a wildlife studies were conducted in 2003 and 2007 that included the collection of white-tailed deer (*Odocoileus virginianus*) from both the TR and SR floodplain. Tissues that were analyzed included muscle and liver. Statistical analyses indicated no significant differences in total 2378-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ) for either liver or muscle tissues between male and females and analysis of age was inconclusive due to small sample sizes. Liver TCDD-EQ concentrations were statistically significant greater than muscle whether based on wet weight or liver normalized values. For both muscle and liver tissues, TCDD-EQ concentrations were significantly greater in deer collected downstream of Midland when compared to deer from the upstream reference location. Principle component analysis (PCA) showed that the congener patterns in deer upstream of Midland were dominated by PCDD congeners while in the downstream location the pattern was dominated by two PCDF congeners, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF. PCA analysis of soil data from upstream and downstream locations indicated a congener pattern that was similar to that observed in deer collected from these locations. Overall, these data indicate that deer accumulate PCDD/PCDF in a manner that is reflective of the concentrations and congener profiles in found in floodplain soils.

**WP267 Chronic Copper Exposure in Three Species of Amphibians: Lethal, Sublethal, and Gene Expression Effects** S. Lance, Univ of Georgia, Savannah River Ecology Laboratory; K. Jones, Univ of Colorado School of Medicine; R.W. Flynn, Univ of Georgia, Savannah River Ecology Laboratory; M. Erickson, Georgia Southern Univ, Biology; T. Tuberville, D. Scott, Univ of Georgia, Savannah River Ecology Laboratory. Chronic exposure to environmental contaminants can cause effects at higher levels of biological organization such as populations and communities. However, due to inherent challenges in assessing these impacts most studies focus on individual survivorship under acute exposure. To assess the long-term effects of contaminant exposure it is critical to examine sub-lethal endpoints, and the potential for organisms to adapt to contaminated environments. We are investigating the relevance of sub-lethal endpoints in amphibians exposed to copper contamination. To date we have examined three species: southern leopard frogs (*Lithobates sphenoccephalus*), southern toads (*Anaxyrus terrestris*), and eastern narrowmouth toads (*Gastrophryne carolinensis*). Overall we have found significantly different sensitivities to chronic copper exposure at the individual, population, and species levels. Leopard frogs are more tolerant to copper and successfully developed to metamorphosis in up to 150 ppb copper. However, survival is negatively impacted at 50 ppb. Southern toads and eastern narrowmouth toads are more sensitive and no larvae have reached metamorphosis at concentrations above 15 ppb. In all studies we reared eggs from clutches of multiple breeding pairs and source populations, and

have found these factors to be significant sources of variation in survival. In leopard frogs we observed significant differences in egg/larval success among breeding pairs, while in southern toads there was a significant effect of source population. To further examine effects of copper on sub-lethal endpoints we are continuing treatments on post-metamorphic southern toads through reproductive maturity to assess effects on time to and size at maturity as well as survival. In addition we are using RNASeq approaches to look at the transcriptional changes that may be related to sub-lethal effects and may contribute to differences in survival and other life history endpoints. Currently we are investigating the effects of copper exposure on the transcriptome of developing southern toads from several individuals from two populations throughout egg, larval, and post-metamorphic development.

**WP268 Development of Molecular Indicators to Track the Effects of Nanoparticle Toxicity in *Arabidopsis thaliana*** L. Tumburu, National Research Council; C.P. Andersen, USEPA NHEERL/WED; M.G. Johnson, USEPA NHEERL/WED, NHEERL/WED; P.T. Rygielwicz, USEPA NHEERL/WED; G.A. King, Dynamac Corporation; J.N. Betts, M. Plocher, Dynamac Corporation, Environmental Protection Agency. The emergence of nanotechnology and incorporation of nanoparticles in consumer products necessitates risk assessment from an environmental and health safety standpoint. To date, very few studies have examined nanoparticle effects on terrestrial species, especially plants. Preliminary results from an *Arabidopsis thaliana* Columbia (Col-0) study performed with titanium dioxide (TiO<sub>2</sub>) nanoparticles (P-25) using five test concentrations indicated reduced cotyledon expansion at 10 ppm and slowed germination at the highest concentration, suggesting that different mechanisms of response may be involved at the two concentrations. To examine the molecular basis for response, a study was conducted to examine how different concentrations of TiO<sub>2</sub> affected different life stages of *A. thaliana*. Dynamic Light Scattering/Electrophoretic Light Scattering (DLS/ELS) technique was used to characterize TiO<sub>2</sub> suspensions (concentrations 5mg/L and 500 mg/L). Particle suspensions were determined to be in the < 100 nm diameter range. *A. thaliana* plants were exposed starting in the seed stage, to weekly suspensions of these mixtures. Subsequently, with the growth of plants, both root and shoot systems were exposed to the mixtures until they reached senescence. Following exposure, five different tissues (cotyledons, roots, leaves flowers, and siliques) at different developmental stages were harvested. Following the extraction of RNA from the tissues, and subsequent reverse transcription to cDNA, and conversion to cRNA, hybridization was performed with commercially available *Arabidopsis* DNA microarrays (Affymetrix) to obtain the tissue-specific transcriptome profile of *Arabidopsis*. The results varied with developmental stage, and showed that while some differences in gene up- and down-regulation were observed between the two concentrations, some differences may be related to differences in particle dispersion rather than concentration. Fully characterizing particle suspensions using DLS as well as other approaches such as Small-Angle X-Ray Scattering [SAXS], X-Ray Diffraction [XRD] was important. The findings of this research will help to elucidate mechanisms of action associated with nanoparticle induced toxicity and provide primary screening information for terrestrial ecological risk assessments.

**WP269 Effects of In Ovo Exposure to an Estrogenic Chemical on Development and Reproduction of Western Fence Lizards** A. Delecki, Oklahoma State Univ, Natural Resource Ecology and Management; L.G. Talent, Oklahoma State University, Natural Resource Ecology and Management. Many tons of pesticides and herbicides are used in the USA each year and some contain chemicals that mimic hormones, including estrogen. Some of these chemicals can persist for long periods in soil where embryonic lizards may be exposed because many lizards bury their eggs in the substrate for incubation. To evaluate the effects of in ovo exposure to estrogenic chemicals on the subsequent reproduction of adult lizards, we administered varying doses of 17 $\alpha$ -ethinylestradiol (0, 0.000001, and 0.001  $\mu$ g/egg) to western fence lizard (*Sceloporus occidentalis*) eggs by microinjection. Eggs were injected 7 days post oviposition and incubated at 26 °C. At hatching, neonates were weighed, measured, and phenotypic sex was determined by secondary sex characteristics. In ovo exposure of 7-day old embryos to 0.001  $\mu$ g 17 $\alpha$ -ethinylestradiol per egg caused all male embryos to develop into phenotypic females. However, as the hatchlings grew to maturity under laboratory conditions, males developed secondary sex characteristics, indicating that compete sex reversal did not occur. When lizards were six months of age, they were separated into heterosexual pairs and placed in

large aquaria to determine if reproduction was affected by estrogen exposure. Lizards exposed in ovo to 0.001  $\mu\text{g}$  17 $\alpha$ -ethinylestradiol per egg did not produce any fertile eggs, while all other groups produced fertile eggs. The copulatory organs (hemipenes) of males that were exposed in ovo to 0.001  $\mu\text{g}$  17 $\alpha$ -ethinylestradiol per egg were underdeveloped. Therefore, it appears that the reproductive systems of males and possibly females were permanently altered by in ovo exposure to ethinylestradiol.

**WP270 Effects of Maternal Dietary Exposure to 17 $\beta$  trenbolone on the Growth and Development of Japanese Quail Embryo** Y. Chen, Virginia Tech, Dept of Fisheries and Wildlife Sciences, USGS Patuxent Wildlife Research Center, USGS Patuxent Wildlife Research Center, Patuxent Wildlife Research Center; N. Karouna-Renier, USGS Patuxent Wildlife Research Center, Beltsville Lab ; P.F. Henry, USGS Patuxent Wildlife Research Center, Patuxent Wildlife Research Center; C. Maddox, Patuxent Wildlife Research Center, USGS, Beltsville Lab ; D.T. Sprague, Patuxent Wildlife Research Center, USGS. In ovo exposure to trenbolone, a potent anabolic and androgenic feed additive used in the cattle industry, can affect sexual development and onset of maturation in Japanese quail (*Coturnix japonica*). The current study focuses on the effects of maternal dietary exposure to 17 $\beta$  trenbolone on quail embryonic development. Adult Japanese quail were paired and treated with 5 doses of 17 $\beta$  trenbolone in feed. Upon commencement of egg laying, fertility (E8) and embryonic development and viability (E15) as well as hatching success were monitored to assess productivity. A subset of eggs were similarly artificially incubated however they were removed after 12 (E12) and 14 (E14) days of incubation. The embryos from these eggs were sacrificed, somatic indices (e.g., body/organ weight; tarsus length) were recorded to assess relative growth and development. Expression of sex steroid responsive genes (Vitellogenin II (VTG), Very low-density apolipoprotein II (apoVLDL II), and ZP1) in liver of these embryos was measured by real-time quantitative PCR. Previous studies in our group revealed trenbolone exposure can alter plasma testosterone concentration as well as the expression of VTG and ZP1 in adults. Such changes in the reproductive system in parents, in addition to the in ovo exposure of trenbolone, are expected to affect the normal pattern of sex steroid regulated gene expression in developing embryos. Findings from the current study are discussed in the context of endocrine disruption during embryonic development.

**WP271 Fate and Effects of Depleted Uranium in Terrestrial Systems** J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center, Research Biologist; J.K. Stanley, S. Brasfield, US Army Engineer Research and Development Center, Environmental Laboratory; A.J. Bednar, US Army Engineer Research and Development Center; D. Meeks, US Army Research and Development Center. Depleted uranium (DU) from the military testing and use of armor-piercing "penetrators" has been shown to accumulate in both soils and sediments; however, little is known about the environmental fate and effects of DU. The purpose of the current study was to assess the environmental fate and effects of various DU geochemical species to a model terrestrial invertebrate, the earthworm, *Eisenia fetida*. To assess the impact of DU on bioaccumulation, growth, reproduction, and cellular stress we conducted a range of acute and chronic exposures. Acute soil studies consisted of a 14 day range finding exposure and soil avoidance bioassay utilizing DU contaminated and spiked soils from multiple Army locations. Additionally, a 72 hour filter paper exposure (n=10) utilizing a DU oxide solution (treatment range: 0-1,000 mg/kg) was conducted to assess dermal uptake. Dermal uptake exposures were coupled with neutral red retention time (NRR) assays and utilized as a biomarker of cellular stress; measurements were conducted utilizing Sigma Tox4 Toxicity Assay (St. Louis, MO, USA). Chronic, 28 day soil exposures were conducted with field collected DU contaminated sand as well as clean soils from military range, spiked with DU oxide solutions (n=3, 0-3,000 mg/kg). Results to date have indicated no significant reductions in earthworm survivorship at soil concentrations of DU up to 16.7 mg DU/kg; the presence of cocoons also indicates the ability of earthworms to reproduce in low levels of DU contamination. The current work represents part of a larger Army research focus area to determine the environmental impact of DU munitions and residues in complex active range environments and to develop methods for the cost effective and environmentally protective management and/or removal of small metallic DU and DU residues from affected soils and sands.

**WP272 Impact of Exposure Level and Condition on the Maternal Transfer of PDE-99 in Zebra Finches** M.L. Eng, Simon Fraser Univ; T.D. Williams, Simon Fraser Univ, Biological Sciences; R.J. Letcher, Carleton Univ, Dept of Chemistry, Environment Canada, Science and Technology Branch, Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, Great Lakes Institute for Environment, Canadian Wildlife Service; J.E. Elliott, Environment Canada, Pacific Wildlife Research Centre, Science and Technology Branch, Environment Canada, Pacific Wildlife Research Centre, Environment Canada, Canadian Wildlife Service. Early life stages in birds are sensitive to environmental conditions, and early exposure to pollutants can have permanent effects on the resulting phenotypes at concentrations much lower than those required to affect adults. Pollutants found in the early embryonic environment (i.e., the egg) are generally accepted to be of maternal origin. Polybrominated diphenyl ethers have become ubiquitous in the environment, and BDE-99 is one of the most common congeners found in wildlife samples, including in bird eggs. The objectives for our study were to identify what effect the level of exposure and the body condition of the mother have on the rate of transfer of BDE-99 from the mother to the egg. To examine maternal transfer of BDE-99, we dosed adult female zebra finches with environmentally relevant levels (0, 25 or 250ng/g bw/day) of BDE-99 for three weeks prior to pairing. 15 females were dosed for each of the three doses. We measured blood levels of BDE-99 in the females on the day they laid their first egg and at clutch completion to estimate the reduction in body burden from egg laying. Blood was also analyzed for lipid levels. A single egg and a single hatchling were collected per female to estimate transfer from the mother to the egg to the embryo. We collected the third egg, as its timing of formation corresponds with the timing of the first blood sample. We found a significant dose response in the concentrations of BDE-99 in all samples (first egg blood sample 0.7, 25.6, or 241.6 ng/g ww; clutch completion blood sample 0.6, 23.0, or 114.6 ng/g ww; egg 4.0, 123.3 or 465.9 ng/g ww; first hatched chick 2.0, 112.2, or 399.4 ng/g ww for the 0, 25, and 250ng/g dose groups, respectively). Maternal plasma BDE-99 concentration significantly decreased from the day the first egg was laid to clutch completion. Maternal lipid status was not significantly correlated with the concentration of BDE-99 found in the eggs.

**WP273 Investigating the Toxicity of Ultraviolet Filter Chemicals and Their Photochemical Degradation Products** S. Suhag, S. Bercovici, A. Kracunas, M. Paulick, L.A. MacManus-Spencer, Union College, Dept of Chemistry. Sunscreens are established commodities of the American lifestyle as indicated by product sales rising from 18 million dollars in 1972 to 500 million dollars in 2005. Sunscreen's ability to protect skin from the sun's harmful ultraviolet rays is credited to ultraviolet filter chemicals (UVFCs), which are organic chemicals ranging from five to twenty percent concentrations in the product. Currently, the Food and Drug Administration regulates the UVFC content in sunscreens and has "red-flagged" those exhibiting toxicity, such as the skin irritating, potentially carcinogenic para-aminobenzoic acid (PABA). Two commonly used and FDA approved UVFCs are octyl methoxycinnamate (OMC, octinoxate), and octyl dimethyl para-aminobenzoic acid (OD-PABA, padimate-O). These and other UVFCs enter the aquatic environment by direct inputs, such as swimming, and indirect inputs via wastewater treatment plants. Several UVFCs have been detected in surface waters and in the muscle and fatty tissue of fish. The increasing use of products containing these chemicals raises concerns about their negative impacts on the environment. UVFC structures are expected to withstand the absorption of sunlight and thus be photostable. However, previous research has revealed that OMC and OD-PABA are susceptible to direct photolysis, forming several degradation products. For example, the photostable products of OMC photolysis include 4-methoxybenzaldehyde, 2-ethylhexanol and cyclodimers of OMC. Current research on OD-PABA's degradation using liquid chromatography – mass spectrometry has revealed demethylated products and some evidence of the formation of PABA. The toxicity of the parent compounds has been studied, but the toxicity and environmental impacts of the photolysis products has yet to be thoroughly investigated. PABA and other photolysis products were tested for toxicity to human skin cells using a Trypan blue assay. Understanding the environmental fate and toxicity of UV filter chemicals and their degradation products will lead to more informed decisions about their use and regulation in personal care products.



**WP274 Lethal and Sub-lethal Effects of Chronic Copper Exposure on Southern Toads, *Anaxyrus terrestris*** R.W. Flynn, D. Scott, Univ of Georgia, Savannah River Ecology Laboratory; K. Jones, Univ of Colorado, School of Medicine; T. Tuberville, S. Lance, Univ of Georgia, Savannah River Ecology Laboratory. Exposure to environmental contaminants is one cause of global amphibian population declines. Contamination is a widespread phenomenon, but most ecotoxicology studies have focused on endpoints based on acute exposures, which underestimate the effects of chronic exposures. For amphibians, aquatic heavy metal contamination is an increasingly common and persistent issue. We have been investigating the effects of chronic aquatic copper contamination on southern toads, *Anaxyrus (Bufo) terrestris*. We are using a combination of laboratory and outdoor mesocosm studies to examine how such contamination affects local populations. In our lab studies we have found that both copper and the source population of the parents significantly affect survival of eggs and larvae. To further this work in a more natural system, we reared toads in outdoor 1500-L experimental ponds. We set up 24 mesocosms in a replicated factorial design to examine effects of source population (N=2) and copper concentration (N=3) on metamorph traits. We placed 100 eggs (20 from 5 different *A. terrestris* breeding pairs) in each mesocosm; for all metamorphs we are determining time to and size at metamorphosis, and for a small subset we are measuring metal body burdens. Finally, we sampled subsets of toads from four different time periods during the mesocosm studies to examine the effects of copper on gene expression patterns. Because eggs came from toads from different populations (with and without previous copper exposure) we will look at differences in the transcriptomics that may account for some of the population-level differences we see in sensitivity to copper.

**WP275 OECD TG223 Avian Oral Dose Test (LD50): Utilizing Passerine Species** T.B. Fredricks, Monsanto, Zoology; M. Christ, Bayer CropScience. Laboratory validation of the OECD TG223 avian oral dose test was previously conducted with bobwhite quail (*Colinus virginianus*) however the utility of this study design has not been determined for passerine species. The OECD TG223 test design uses multiple sequential testing stages resulting in fewer birds (e.g., 39 birds) needed to estimate the LD50, confidence intervals, and slope. The OECD TG223 is in contrast to the USEPA guideline study (OPPTS 850.2100) that uses up to 60 birds in a classic single-stage dose-response design. The current study provides information on the usefulness of the OECD TG223 oral dose test to assess the toxicity of two pesticide products on the canary (*Serinus canaria*) and zebra finch (*Taeniopygia guttata*). The birds were dosed with MCPA Acid and Isazophos, which were previously used for the OECD TG223 validation with bobwhite quail. The compounds are characterized as producing low toxicity values with a steep slope (MCPA Acid) and high toxicity values with a moderate slope (Isazofos). Both species were capsule dosed with each chemical at multiple stages with the aid of a Sequential Design Calculator (SEDEC) to determine the LD50, confidence intervals, and slope. Endpoints determined for each species and each test stage included: sub-lethal effects, mortality, bodyweight, and food consumption. The LD50 values for each chemical were unreliable as regurgitation occurred following the oral dose of each species. This research provides further test development and potential information supporting standardization of regulatory requirements for passerine testing in the United States combined with the conscientious vertebrate testing standards in the European Union.

**WP276 Potential for Bioremediation Through Formation and Fate of Metal Rich Granules in the Terrestrial Environment** M. Ansley, US Army Engineer Research and Development Center; S. Brasfield, US Army Engineer Research and Development Center, Environmental Laboratory; J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center, Research Biologist; J. Seiter, US Army Corps of Engineers, Research Physical Scientist; B. Little, The Ohio State Univ, ESGP; R. Jones, US Army Engineer Research and Development Center; R. Lanno, Ohio State Univ, Dept of Evolution, Ecology, and Organismal Biology. With respect to US military installations, lead (Pb) is the most common metal contaminant found on small-arms Army ranges. Currently, risk assessors assume 100% Pb bioavailability based on soil concentrations, although many terrestrial invertebrates are capable of rendering metals toxicologically inactive through the formation of metal rich granules (MRGs). In earthworms, metals can be sequestered through chloragosome tissue and metallothioneins (specialized proteins); once critical body burdens are reached, additional accumulation and toxic response to metals

is diminished. While MRGs are thought to be trophically unavailable to invertebrates and predators, there is little known regarding the fate of MRGs once released from the exposed organism. The current research characterizes the microstructure of MRGs formed by the earthworm, *Eisenia fetida* and determining the potential for microbial degradation to remobilize Pb that has been previously bound in MRGs. If liberated by bacterial degradation, bound Pb may become newly available to invertebrates and their predators. We performed a series of soil exposures utilizing Pb spiked field soils and chronic earthworm exposures. Earthworms were exposed to soils spiked with 4000 mg/kg Pb for a six week period and fractionated utilizing differential centrifugation and digestion to separate whole-animal tissue from MRGs. Once extracted, MRG composition and concentration was analyzed utilizing synchrotron based analysis at Argonne National Laboratories (Argonne, IL). Synchrotron imaging provided multi channel analyzer plots (MCA) which revealed localized MRG hotspots within the samples confirming the presence of not only lead, but also the binding of additional metals such as zinc and iron. Additionally, synchrotron techniques were utilized on cross-sections of earthworms to determine the presence and concentration of Pb across a gradient of dermal and gut tissue. MCA plots of cross sections revealed a wide dispersion of Pb inside the gut wall, suggesting the presence of un-bound Pb in addition to MRG formation.

**WP277 Repeatable Among Individual Variation in Bioaccumulation of Mercury-dosed Zebra Finches** K. Buck, The College of William and Mary, Biology; C. Ramos, D. Cristol, J. Swaddle, The College of William and Mary. Mercury is a ubiquitous ecotoxin with numerous detrimental effects on birds. Although mercury accumulates to different levels in different bird species, little is known about the natural variation of within-species mercury accumulation. Zebra finches (*Taeniopygia guttata*) were maintained in controlled environmental conditions on a standardized diet containing either 0.0, 0.1, 0.4, 0.5, or 1.0 mg/Kg methylmercury. Within treatments, individual zebra finches consistently differed in the amount of mercury accumulated in their blood. Among-individual variation in blood mercury greatly exceeded within-individual variation across all mercury dose treatment groups. Among-individual differences in blood mercury accumulation may reflect an underlying genetic basis for mercury mitigation, potentially allowing evolutionary adaptation to environmental mercury contamination if this variation is heritable (genetically or epigenetically). As part of an ongoing effort, the narrow-sense heritability of mercury accumulation in zebra finches maintained on diets containing 0.5 and 1.0 methylmercury was calculated using parent offspring-regressions and full sibling comparison. More research, however, is necessary to understand gene expression mechanisms underlying mercury tolerance in birds.

**WP279 Selecting Experimental Endpoints to Measure Androgenic Endocrine Disruption in Avian Models** P.E. Henry, USGS Patuxent Wildlife Research Center, Patuxent Wildlife Research Center; M.R. Bakst, USDA – ARS, Animal Biosciences and Biotechnology Laboratory; V.G. Akuffo, USDA, ANRI ABB; D. Sprague, Y. Chen, N. Karouna-Renier, USGS – PWRC. Although progress has been made in assessing effects of environmental contaminants that disrupt estrogenic-related processes in wildlife, far less is known about measuring disruption when the chemical is androgenic or anti-estrogenic. Along with the increase in release of emerging chemicals, personal care products, pharmaceuticals, and agricultural feed conversion synthetic hormone mixtures, is the increased detection of anti-estrogenic and androgenic chemicals. A rational basis is needed for the development and selection of appropriate endpoints for testing and assessing androgenic exposure and/or effect. Starting with the avian endocrine system and focusing on receptors, tissues, and organ changes related to native testosterone or its metabolites, we are evaluating the efficacy of potential endpoints. Using findings obtained from a study in which Japanese quail were fed an anabolic androgen disrupting synthetic steroid, 17 $\beta$  trenbolone, we discuss the use of markers such as onset of foam production, plasma testosterone, sperm activity relative to the developing blastodisc, and the viability of sperm storage tubules – markers that may reflect alterations in fertility of birds caused specifically by androgenic endocrine disrupting chemicals.

**WP280 Studies on the Phyto-toxicity and Accumulation of Heavy Metals Using *Capsicum annum* L.** M. Kim, Korea Univ; J. Son, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; H. Mo, Korea Univ, Division of

Environmental Science and Ecological Engineering; Y. Kim, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; Y. Lee, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; K. Cho, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences. Heavy metal contamination of soils may deleterious effect on human and environmental health through a food chain. In this respect, plants have been considered a route that transfers the heavy metals from soil to herbivorous insects. As a preliminary study of the metal accumulation from soil to invertebrate, phyto-toxicity test about each metal using *Capsicum annuum* L. was implemented in this experiment. A pot experiment was carried out to evaluate the effects of arsenic, cadmium, lead and zinc on biomass, shoot and root length, chlorophyll, and heavy metal content of *Capsicum annuum* L. grown in artificial substrates for three weeks. Artificial substrates consist of potting mix and OECD artificial soil which make up with sand, silt and clay with 75, 20, 5% respectively. Using the measured metal concentrations in soils and plant part of the pepper, bio-concentration factors and translocation factors (from root to shoot) were calculated. In the majority of cases, application of metal increased both total and bio-available forms of metals in the soils and plant growth parameters were decreased. Our results showed that pepper plant could store a high concentration of heavy metals in the root rather than shoot.

**WP281 The Effects of Glyphosate and Aminopyralid on Artificial Plant Communities** T. Pfleger, M. Blakeley-Smith, USEPA WED; G. King, Dynamic, Inc; H. Lee, D. Olszyk, USEPA WED; M. Plocher, Dynamic, Inc. The USEPA has responsibility for registration of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The potential adverse effects of pesticides to nontarget terrestrial plant communities are a concern that must be addressed in the pesticide registration process. The protection of nontarget terrestrial plants requires a tiered approach using two single species greenhouse tests with an increasing level of test sophistication being required if a compound fails at the preceding tier. This can culminate in field testing which currently is not well-defined. Our objective was to develop a simple, economical, and geographically flexible field test with ecologically significant endpoints. Three plant species native to Oregon: *Clarkia amoena* (Farewell to spring), *Prunella vulgaris* (Self-heal), *Festuca roemerii* (Roemer's fescue) were grown together along with a fourth introduced species, *Cynosurus echinatus* (Bristly dogtail grass). The experiment was replicated at two research farms within the Willamette Valley, Oregon. Round up Original (41% glyphosate) was applied at 0, 0.01, 0.1, and 0.2 x FAR (Field Application Rate) (32 fluid oz/acre (2.34 l/ha) roundup). Milestone (40.6% aminopyralid) was applied at 0, 0.037, 0.136, and 0.5 x FAR (7 fluid oz/acre (.51 l/ha) milestone). One third of the plots received a single application, 1/3 received two applications and 1/3 received three applications at the above listed rates. The control was a no spray treatment. Herbicide applications were two weeks apart. Plant height and width, in two perpendicular directions, were measured every two weeks during the growing season. Periodically, buds and flowers were counted and seeds were collected. Differences between sites were minor. Increasing the number of applications had less than an additive effect. Plant volume decreased with increasing glyphosate concentration. *Clarkia* died at all concentrations of aminopyralid while *Festuca* and *Prunella* increased in volume. *Cynosaurus* increased in volume at the lowest concentration of aminopyralid while decreasing at the highest concentrations. Flowering was delayed in *Clarkia* when exposed to glyphosate. The results indicate that simple field tests can be successfully performed to investigate the ecological effects of herbicides on nontarget plant interactions. We found that simple physiologically based greenhouse toxicity testing is not a reliable predictor of ecological outcomes in the field.

**WP282 The Effects of Perfluoroundecanoic Acid (PFUnA) Injections on Developing Japanese Quail (*Coturnix japonica*)** A.N. Iwaniuk, Univ of Lethbridge, Neuroscience; K. Dean, Univ of Lethbridge, Neuroscience, Univ of Maryland, post-doctoral research fellow. Perfluorinated chemicals are virtually ubiquitous in the environment and are found in a wide range of wildlife species. The potentially harmful effects of chronic exposure to low levels of these chemicals is, however, poorly understood. Furthermore, most laboratory studies focus on relatively high doses of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and very little is known about the effects of environmentally relevant levels of exposure to other perfluorinated chemicals. Here, we focus specifically on perfluoroundecanoic acid

(PFUnA), the second most abundant perfluorinated chemical found in the eggs of wild birds. PFUnA was injected at low, environmentally relevant doses (0.05-3.0 µg/g) into Japanese quail (*Coturnix japonica*) eggs. The eggs were then incubated and necropsies conducted on day of hatching. We observed a significant increase in mortality rate across all of our PFUnA doses relative to both controls and PFOS injected eggs. PFUnA exposed hatchlings had significantly more developmental abnormalities than control eggs, but did not differ in hatchling body weight or other measures of body size. Lung, brain and adrenal weights did not differ between PFUnA treated eggs and controls. In contrast, testis, ovary+shell gland and bursa weights were lower and yolk and gastrointestinal tract weights were higher in PFUnA treated eggs than in controls. Thyroid, heart and liver weights were lower only in some PFUnA treatment groups. In addition, to organ weights, we also conducted thyroxine (T4) assays and examined brain morphology across treatment groups. Overall, we conclude that exposure to environmentally relevant levels of PFUnA affects egg survival and multiple organ systems of hatchlings and could therefore have deleterious effects on wild populations.

**WP283 The Influence of Environmental Dynamics on Mercury Concentrations in the Florida Panther (*Puma concolor coryi*)** A. Brandon, Florida Gulf Coast Univ; M. Cunningham, D. Onorato, Florida Fish and Wildlife Conservation Commission; D. Jansen, National Park Service; D. Rumbold, Florida Gulf Coast Univ. The Florida panther (*Puma concolor coryi*) is a federally listed endangered sub-species of puma located in the Everglades and Big Cypress ecosystems of southern Florida. Environmental stressors, including environmental contaminants, low genetic variability and habitat loss may have contributed to the decline of this sub-species. Hair and blood were collected for analysis through live capture and liver, brain, muscle and kidney were collected during necropsies of salvaged dead cats. Concurrently, other metrics including body condition, presence of congenital defects, blood chemistry analysis and reproductive success were also assessed as part of a larger investigation on health and fecundity of the population. Evaluated here were the spatial and temporal patterns in hair-Hg and blood-Hg over the period of record (1978-2007) and the potential influence of hydroperiod as an explanatory variable as it relates to methylation potential and habitat quality for prey species. All collected hair (n=478) and blood samples (n=338) had measureable concentrations of mercury, ranging from 0.086 mg/kg to 100 mg/kg and 0.009 mg/kg to 5.3 mg/kg, respectively. These data demonstrated that while average Hg levels have decreased in panthers in most areas, some individuals continued to attain high mercury burdens. As an endangered species suffering from numerous stressors, these maximum exposure concentrations remain a concern. These changes in tissue-Hg concentrations were well correlated with variations in hydroperiod identified among the study regions. Regions experiencing repeated wetting and drying cycles and long hydroperiods were home to individuals with elevated tissue-Hg concentrations. Hydroperiod appears to be a significant factor in prey species availability for cats as well as an influence on methylation rates in these areas. Habitat management to support large populations of ungulate species as prey items for Florida panthers is an important part of decreasing their reliance on aquatic food web items and lowers their risk of adverse health effects as a result of exposure to Hg.

**WP284 Toxic Effect of Insect Growth Regulators (IGRs) on Biological Trait of *Paranura rosea* (Collembola)** Y. Lee, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, Environmental Science and Ecological Engineering; J. Son, Y. Kim, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; M. Kim, Korea Univ; K. Cho, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, # 407, Division of Environmental. It is known that insect growth regulators (IGRs) have lower toxicity than conventional insecticides against vertebrates. Although IGRs have a selective effect on target organisms, they may exert a more selective effect on non-target organisms. In this study, the toxic effects of three IGRs (teflubenzuron, fenoxycarb, tebufenozide) on biological traits of *P. rosea*, Collembola, were assessed in the OECD artificial soil under two different exposure conditions, one was exposed in the bulk soil and the other was exposed in the compacted soil which unidirectional force was applied to the soil surface. Several biological traits, such as survived adults, egg production, hatched juvenile and molting, were examined after 28 days exposure to three IGRs. The toxicity of all the IGRs on the survival and juvenile production of *P. rosea* in the bulk system

was more toxic than that of the compact system. Among them, the chitin synthesis inhibitor, teflubenzuron, was most toxic chemical and it is toxic for *P. rosea* at concentration that are probably close to environmental levels. Juvenile hormone agonist, fenoxycarb, was more toxic for juveniles and egg relatively than others. Moreover, the hatching rate and molting frequency of *P. rosea* was decreased in a concentration dependent manner. These results suggest that the IGRs exhibit significant impacts on the biological traits of non-target organism *P. rosea* and its toxic effects are differently assessed depending on the exposure conditions.

#### WP285 Toxic Sulfur Compounds from the Plant *Chorispora tenella*

**O.R. Arnold**, Colorado State Univ, Center for Environmental Medicine, Colorado State Univ, Environmental and Radiological Sciences; H.S. Ramsdell, Colorado State Univ, Center for Environmental Medicine  
*Chorispora tenella* is an introduced species in North America and has been described in at least 31 states including all of those west of the Mississippi and 3 Canadian provinces. It is known by several common names, including blue mustard, crossflower and purple mustard. It is among the *Brassicaceae* species that have been blamed for causing toxic effects in animals. Toxicity of plants in the mustard family is generally attributed to the products of enzymatic hydrolysis of glucosinolates. Additionally, digestible sulfur can be toxic to ruminants when converted to hydrogen sulfide in the rumen. As little phytochemical information is available for *C. tenella*, we have investigated potentially toxic constituents of the plant. Samples were collected in the spring in the vicinity of Fort Collins, Colorado. Plant material was homogenized in pH 7.5 citrate/phosphate buffer and incubated at room temperature. This autolysis process would be akin to what would happen should a grazing animal eat the plant. The volatile products were collected in an overlying layer of pentane in the sealed flask. The pentane extracts were analyzed by gas chromatography and were found to contain allyl isothiocyanate, propyl isothiocyanate and butyl isothiocyanate. Liver, kidney and thyroid toxicity have been associated with allyl isothiocyanate exposure. The other isothiocyanate products of *C. tenella* autolysis may also contribute to adverse effects if the plant is consumed by animals. The aqueous slurry was divided for dry weight and total sulfur content determination. Total sulfur was measured by a standard AOAC digestion modified to measure the resulting sulfate by a turbidimetric method. The sulfur content of the *C. tenella* samples was found to exceed 0.3% of plant dry weight. This level is sufficient to be of concern for cattle also consuming water with elevated sulfate concentrations, as is commonly found in ground water in eastern Colorado and the northern Great Plains.

and 0.76 µg insecticide/g body for permethrin, naled, and dichlorvos, respectively. In general, time needed for caterpillars to become butterflies appeared to be longer for permethrin exposure than for control. Based on observation of insecticide residues in the field after application, caterpillars are at risk of being exposed to permethrin and naled via the dietary route. Results of this study suggest that protection of non-target butterfly species should be taken into consideration when applying mosquito control insecticides in the Florida Keys and in other areas of the US.

#### WP286 Toxicity of Mosquito Control Insecticides to Florida Native

**Caterpillars via Ingestion** **T.C. Hoang**, Florida International Univ, Southeast Environmental Research Center, Dept of Environ Study, Loyola Univ Chicago, Center for Urban Environmental Research and Policy; G. Rand, Florida International Univ, Ecotoxicology & Risk Assessment; R. Frakes, US Fish and Wildlife Service. Mosquito control insecticides have been applied frequently in the Florida Keys. Non-target organisms such as butterflies are at risk of being exposed to mosquito control insecticides. Toxicological research indicates that larval stages of butterflies (i.e., caterpillars) are sensitive to mosquito control insecticides via topical dermal contact. However, during mosquito control insecticide application, caterpillars can and will be exposed to chemicals via ingestion of contaminated food (host plant). This study characterized the effects of permethrin, dibrom (naled), and the naled metabolite (dichlorvos) to three Florida native caterpillars (4<sup>th</sup> instar stage *Junonia coenia* (common buckeye), white peacock (*Anartia jatrophae*), atala hairstreak (*Eumaeus atala*)) using an ingestion method. Host plants were dipped in either permethrin, naled or dichlorvos solutions for approximately 1 minute and then air-dried for approximately 1 minute before feeding caterpillars. Survival was determined 24 h post exposure. After 24 hour exposure, contaminated host plants was removed (if left) and surviving caterpillars were fed fresh untreated host plants daily up to pupation occurred (5 days). The studies were conducted for more than two weeks until caterpillars pupated to butterflies. Time needed for caterpillars to pupate to butterflies and pupation hatching efficiency were characterized. Results of this study indicated that dietary exposure of mosquito insecticides produced toxicity to caterpillars. White peacock caterpillars were most sensitive to permethrin. The 24-h LD 50s for white peacock caterpillars were 0.41, 0.62, and 1.80 µg insecticide/g body for permethrin, naled, and dichlorvos, respectively. Buckeye caterpillars appeared to be more sensitive to naled than dichlorvos and permethrin. The 24-h LD50s for Buckeye caterpillars were 0.24, 0.33,



**RP001 An In Situ Thermal Extraction and Detection System for Rapid, Accurate, Quantitative Analysis of Environmental Pollutants in the Subsurface** P. Antle, Tufts Univ; A. Robbat, Tufts Univ, Chemistry. The characterization of environmental pollutants is highly important for not only understanding their fate and behavior, but also predicting the potential long-term impact and taking necessary clean-up measures. With evaporation, dissolution, and sorption partitioning some pollutants and pollutant components and microbial degradation transforming others, distinguishing the role each process plays is important from an assessment and cleanup perspective. Previous research has led to the determination of those mass spectral patterns needed to target PAH, PASH, and their alkylated homologs (important biomarkers for petroleum hydrocarbons) lost to evaporation, dissolution, and degradation. This powerful characterization method loses a level of utility, however, if hampered by traditional sampling and analysis procedures, which are slow, expensive, and inefficient. In contrast, real-time chemical analyses better capture the extent and nature of the contamination because areas that are contaminated are highly sampled whereas areas shown to be clean are less sampled. To this end, we at the Tufts Univ Center for Field Analytic Studies and Technology have developed an in situ sample collection system that provides on-line, real-time detection of environmental pollutants, including fossil fuels and their byproducts, chlorinated solvents, biphenyls (PCBs), and pesticides without bringing sample to the surface. During real-time analysis, the system's heated membrane inlet probe (MIP) is driven into the subsurface, extracting and desorbing organics from groundwater and soil, respectively, and a high temperature transfer line (HTTL) transports said organics to a photoionization detector (PID) for continuous depth profiling. When the PID signal spikes, probe advancement is halted, and flow is diverted to cryotrap for preconcentration, followed by thermal desorption and analysis by a portable gas chromatograph/mass spectrometer (GC/MS). As the system is integrated with VOC and SVOC calibration units, this GC/MS analysis allows for not only detection, but also identification and semi-quantification of all subsurface organics. This is the only technology that can completely, unambiguously profile the subsurface as the probe is advanced from the surface to bedrock.

**RP002 Regional and National Ambient Background Investigations for PAHs and Metals** S. Emsbo-Mattingly, New Fields Environmental, Forensics Practices, LLC; W. Swanson, CDM. Ambient background generally refers to the presence of anthropogenic contaminants in various environmental media cumulatively generated by point and non-point sources and distributed by global, regional, and local processes. The understanding of ambient background in soils, in particular historic fill soil/brownfields, has received a great deal of attention since 2000. Research consortia including USGS, EPRI, Civic Agencies, and affiliated consultants are producing valuable reference data sets for demonstrating the influence of soil type and land use on contaminant analyte concentrations. This presentation provides 1) an update on regional and national efforts to better quantify ambient background and 2) technical strategies to differentiate ambient background from point sources, such as former MGPs. Studies of urban background in Illinois and Massachusetts provide concentration benchmarks for comparison to surface soils based on land use (i.e., rural, suburban, or urban) and soil type (i.e., fill present or absent). The presence or absence of fill is particularly important in residential, commercial, and industrial areas around MGPs as demonstrated by a comparison of the Illinois (fill absent) and Massachusetts (fill present) datasets. This presentation will also discuss recent efforts on the part of the USGS to conduct a comprehensive national background study that includes a greatly expanded list of metals, PAHs, hydrocarbons, and other constituents. Preliminary results from the New England phases of this project will be presented.

**RP003 Distinguishing the Source of a BTEX Groundwater Plume Using Multiple Lines of Evidence** C.B. Tuit, Gradient, Associate; E. Butler, Gradient. In 2005, a Benzene, Toluene, Ethylbenzene, and Xylene (BTEX), chlorinated solvent, and Freon groundwater plume was discovered adjacent to a former refinery site. The contamination, including the chlorinated compounds, was initially attributed to the former refinery laboratory; however, further investigation suggested that an aerosol packaging company operating on the site of the former refinery laboratory may have contributed. Spatial analysis of the chlorinated solvent and Freon plumes showed two distinct sources originating from the site of the former refinery laboratory. Hydrocarbon fingerprinting and compound specific isotope analysis of benzene proved that the hydrocarbons associated with the chlorinated solvent and

Freon plumes had a different source than the hydrocarbons under the adjacent refinery property. Historical groundwater plume maps were developed that distinguished the refinery and non-refinery contaminant plumes. Temporal analysis of the first detections of the chlorinated compounds, and travel time analysis of the plumes indicated that the chlorinated solvent and associated hydrocarbon plume post-dated refinery operations at the former refinery laboratory site. These multiple lines of evidence were able to identify the aerosol packaging company as the source of both the chlorinated solvents and the BTEX contamination.

**RP004 Fingerprint and Weathering Characteristics of Stranded Oils After the Hebei Spirit Oil Spill** U. Yim, Korea Ocean Research and Development Institute, Oil and POPs Research Group; S. Ha, J. An, S. Ha, S. Hong, M. Kim, J. Jung, W. Shim, Korea Ocean Research and Development Institute. After the *Hebei Spirit* oil spill in December 2007, mixtures of three kinds of Middle East crude oil were stranded along the 375 km coastlines of West Coast of Korea. Stranded oils from 19 stations covering three oiled Provinces were monitored for their identity and weathering status. Weathering model results right after the spill indicated evaporation as dominant process and were in line with sequential changes of chemical composition. In the early stages of weathering, half-life of spilled oil was calculated as 2.6 months. Tiered fingerprint approaches revealed that two samples did not match but the other samples had same fingerprint with spilled sources. Even double ratio using alkylated phenanthrenes and dibenzothiophenes showed the effects of weathering 8 months after the spill. However biomarker compounds provided defensible fingerprint and source allocation information. Weathering status of stranded oil was evaluated using composition profiles of saturated hydrocarbons, polycyclic aromatic hydrocarbons and various weathering indices. Most of samples collected 8 months after the spill were categorized as advanced or extreme weathering stages. Gradual changes of hydrocarbon compositions in the residual oils according to the weathering emphasized the adapted ecotoxicological approaches.

**RP006 14C-labeling of Functionalized Multi-walled Carbon Nanotubes (MWCNTs) and Distribution in Fish Tissues** Y.D. Soubaneh, Univ of Quebec, Rimouski, ISMER; E. Pelletier, Institut des Sciences de la Mer de Rimouski, Université du Québec À Rimouski, Univ of Quebec, Rimouski, ISMER/UQAR, Univ of Quebec, Rimouski, ISMER; C. Rouleau, Institut Maurice-Lamontagne; I. Desbiens, Univ of Quebec, Rimouski, ISMER. The lack of solubility of carbon nanotubes (CNTs) in all organic solvents and in water severely limited the use of CNTs in monodisperse solutions and emulsions, but chemical techniques to modify CNTs are progressing rapidly toward surface functionalization of nanotubes and extend their applications to engineering and biomedical applications. Commercial applications of functionalized carbon nanotubes (fCNTs) are expanding rapidly, although the potential risk of this new class of nano-compounds to exposed aquatic organisms is yet little known. Radio-labelling techniques to investigate the distribution and kinetics of toxic metals and organic compounds have been used intensively in biomedical world as well as in environmental organisms. We report in this work a wet chemistry method to functionalize and 14C-label water-dispersible multi-walled carbon nanotubes (MWNTs). An amidation step allows the labeling of these fCNTs by grafting on 14C-labeled ethanolamine. The fCNTs were fully characterized by elemental analysis, electron dispersive X-ray, transmission electron microscopy, thermogravimetric analysis, and Raman and FTIR spectroscopy. An uptake experiment was carried out with Arctic char (*Salvelinus alpinus*) using water dispersed 14C- fCNTs to assess biodistribution in fish tissues using whole body autoradiography. Radio-labeled fCNTs were mainly observed in the fish head and apparently clogging head canals and gills with possible consequences on fish disability.

**RP007 CdSe QD-induced Morphological and Functional Impairments to Liver in Mice** W. Liu, Univ of Pennsylvania Medical School, Dept of Microbiology; Q. Chen, S. Zhang, L. Wang, C. Zhang, S. Liu, G. Jiang, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Quantum dots (QDs), as unique nanoparticle probes, have been used in in vivo fluorescence imaging such as cancers. Due to the novel characteristics in fluorescence, QDs represent a family of promising substances to be used in experimental and clinical imaging. Thus far, the toxicity and adverse health effects from exposure to QDs are not recognized and are publicly

concerned. To assess the biological effects of QDs, we performed a variety of in vivo and in vitro toxicological assays. Results from the present study demonstrated that QD particles could readily spread into various organs, and liver was the major organ for QD accumulation upon in vivo exposure. QDs caused significant hepatotoxicity as reflected by morphological alternation to the hepatic lobules and increased oxidative stress. QDs induced dramatic hepatic toxicity in vivo and in vitro, much greater than that induced by cadmium ions at a similar or even a higher dose. The mechanism underlying QD-mediated biological influences might derive from the toxicity from QD particles themselves and from free cadmium ions liberated from QDs as well.

**RP008 Influence of Meteorological Factors on Submicron Particles in the Urban Atmosphere in Hangzhou** L. Jian, Y. Zhao, Curtin Univ, Curtin Health Innovation Research Institute; Y. Zhu, Hangzhou Traffic Administration and Control Centre. With rapid economic development, the size of road vehicle fleet has increased dramatically in China. In order to characterise the impact of meteorological factors on the emission of submicron particles from vehicles in the urban atmosphere, a model study was conducted in Hangzhou, a fast-developing contemporary city located along the Southeast coast of China. An autoregressive integrated moving average model was used to explore the effects of the meteorological factors on the ultrafine particle (UFP) and particulate matter 1.0 (PM<sub>1.0</sub>) concentrations. Results showed that the average UFP concentration was 45,805 (10,428–147,350) particles cm<sup>-3</sup> and the average concentration of PM<sub>1.0</sub> was 217 (30–506) µg m<sup>-3</sup> during the survey period. While relative humidity (p=0.011) was positively correlated with the UFP concentration, barometric pressure (p<0.001) and wind velocity (p=0.003) were inversely correlated with the UFP concentration. Relative humidity (p=0.022) and wind speed (p=0.023) were also significant predictors of the PM<sub>1.0</sub> concentration. Results from this study suggest that meteorological factors should be taken into consideration in interpretation of air monitoring results for submicron particles as unfavourable meteorological factors may play a role in the formation of new air pollutants and change the ability of the atmosphere to scatter/disperse air pollutants. This study provides first hand information on UFP and PM<sub>1.0</sub> emission levels and a model that can be used in future with large scale time series data to predict the impact of meteorological factors on submicron particle concentrations in the urban atmosphere.

**RP009 Tracking Radiolabeled Silver Nanoparticles in Fish Organs and Tissues** E. Pellerier, Institut des Sciences de la Mer de Rimouski, Université du Québec À Rimouski, Univ of Quebec, Rimouski, ISMER/UQAR, Univ of Quebec, Rimouski, ISMER; I. Desbiens, Univ of Quebec, Rimouski, ISMER; C. Rouleau, Institut Maurice-Lamontagne; D. Bussolaro, Universidade Federal de Parana, Departamento de Biologia Celular. Manufacturers started to introduce nano-sized silver materials into consumer and medical products in early 2000 and over 250 consumer products are claimed to contain silver nanomaterials. Recent progress in our laboratory includes the synthesis of 110mAg labeled nanocomposites (size = 15–60 nm) highly dispersible in natural waters, and the use of a homogeneous dispersion of these nanoparticles to study the uptake and distribution of silver nanomaterials in tissues of bivalves (scallop and mussel) and fish. Results of the exposure of salmonid, *Salvinus alpinus*, to radio-labeled silver nanoparticles (AgNp) using whole body autoradiography are reported. Fish exposed to AgNp from water showed a migration of nanoparticles from gills (6 h after exposure) to liver and intestine (5 days later). Autoradiograms taken 11 days after exposure indicated the presence of radio-labeled silver in head lateral canals and blood system. Fish exposed to food contaminated with AgNp showed a migration of AgNp from stomach (24h after force feeding) toward intestine, spleen and liver 8 days after ingestion. Aggregated AgNp were observed in liver tissues by transmission electron microscopy confirming observations done by autoradiography. Results highlight a slow depuration of labeled AgNp taken from water and food with a tissue distribution different from ionic silver taken also from water and food. Results also indicate a probable oxidation of AgNp to ionic Ag and a distribution in blood system with possible incorporation in bones.

**RP010 Use of the Plant Bioindicator *L. multiflorum* for Ecological Risk Analysis of Air Emissions** R.d. Magali, Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus, Environmental; K. Tallini, Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus, Biotecnology;

M.T. Raya-Rodriguez, Federal Univ of Rio Grande do Sul, Ecology Center. Ecological Risk Analysis (ERA) is a tool used to assess the sustainability of ecosystems, allowing for a more accurate assessment of actual risk to ecological receptors affected by contaminants (Junger, et al., 2005, Sousa, 2005; Nakagome, et al., 2006; Arias, et al., 2007; Niemeyer, 2007, Froehmer & Martins, 2008, Parkhurst, 1996). It is a scientific methodology for quantifying or perceiving the adverse effect risks of a pollutant on the environment (Mines & Lackey, 2009). The study was conducted in the vicinity of an oil refinery (January 2005 to March 2008) in Esteio (29°49'16" S 51°08'09" O), Rio Grande do Sul, Brazil. The aim was to develop and implement an ERA model in order to classify the risks associated with air emissions on the basis of using the bioindicator *Lolium multiflorum*. The latter associated the information from the effect of the stressors, derived from the atmospheric emissions of sulfur compounds, in the receptors, that is, in the tissue of the bioindicator. The percentage of accumulated sulfur was evaluated in the bioindicators, classified, in this study, as a response variable. This was integrated with the data of the SO<sub>2</sub> concentration in the atmosphere, which gave rise to the explanatory variables, toxic potential unit (TPU) and toxic exposure unit (TEU). TPU was defined as the quotient between the average concentration of SO<sub>2</sub> that exceeded the primary air quality standard and secondary air quality standard (CONAMA 03/90), indicating environmental concentrations of SO<sub>2</sub> that can affect the growth of the bioindicators through chronic effect. TEU was defined as the product of TPU and the exposure period of the bioindicators to a higher concentration than the primary air quality standard, indicating SO<sub>2</sub> concentrations that can affect bioindicators through acute effect. The classification TPU < 1 unlikely ecological risk and TPU > 1 potential adverse effects were considered as evidence of risk. On the basis of the environmental quality data (Crittenden & Read, 1979; CONAMA 03/90; USEPA, 1989, Tarazona, 2000, Klumpp, 2001) the classes of ecological risk were established (low, medium and high). The integration between the explanatory and response variables on the basis of multivariate statistical correlation analysis with the use of SPSS software, version 13.0 for Windows, showed evidence of risk to vegetation surrounding the refinery when exposed to TPU greater than the unit.

**RP011 Evaluating the Potential for Endocrine Disruption in Urbanized Aquatic Environments: A Multi-dimensional Assessment** H. Schoenfuss, St. Cloud State Univ, Aquatic Toxicology Laboratory, St. Cloud State Univ, Dept of Biological Sciences MS-273; T. Minarik, Metropolitan Water Reclamation District of Greater Chicago; D. Martinovic-Weigelt, Univ of St. Thomas; M.M. Schultz, College of Wooster, Dept of Chemistry. Urban aquatic ecosystems are often overlooked in toxicological studies even though they serve many functions and often sustain fish populations despite large-scale habitat alterations. However, urban fish populations are likely exposed to a broad range of stressors, including endocrine active compounds that have been found to affect anatomy and reproduction of fish in other studies. Over a two-year study period we have collected and assessed quarterly water samples from 45 locations in the Greater Metropolitan Area of Chicago, IL, for total estrogenicity and a selected subset of samples for analytical chemistry. This effort was coupled with wild- and caged fish studies assessing indicators of endocrine disruption such as vitellogenin induction in male fish and histopathological changes in over 3,000 fish. Our findings indicate that treated effluent from municipal wastewater treatment plants contributes a locally predictable amount of estrogenic activity to its receiving waterways. However, total estrogenicity, measured in vitro, and resultant biological effects are often driven by other, highly-variable urban sources of contamination including runoff and combined sewer overflows that require additional study. The comprehensive assessment used in this study is well suited to understand how endocrine active compounds enter the urban environment and may affect resident fish populations. Results from this study are providing the first system-wide assessment of the potential for endocrine active compounds to interfere with sustainable fish populations in an urban environment. Funding by the Metropolitan Water Reclamation District of Greater Chicago.

**RP012 Tracing the Exposure and Effects of Urban Pollutants to an Aquatic Songbird in UK Rivers** C. Morrissey, Univ of Saskatchewan, Biology; D. Stanton, Cardiff Univ, School of Biosciences; C. Tyler, Univ of Exeter, School of Biosciences; J. Newton, Scottish Universities Environmental Research Centre (SUERC), NERC Life Science Mass Spectrometry Facility; G. Pereira, Centre for Ecology and Hydrology, Lancaster Univ; S. Ormerod, Cardiff Univ, School of Biosciences. Rivers in urban locations

across south Wales in the United Kingdom now show widespread recovery from gross pollution associated with wide-scale industrial development during the 20<sup>th</sup> century. Substantial improvements in regulation of discharges over the last 2 to 3 decades have permitted recolonization of river organisms that can now persist in these environments but little is known about the stability or viability of their breeding populations. Organisms that recolonize recovering locations are exposed to complex mixtures including endocrine disrupting chemicals frequently found in treated sewage effluent and urban runoff. This project aimed to identify the movement of such compounds through riverine food webs to top avian predators, Dippers (*Cinclus cinclus*), using stable isotope tracers and vitellogenin biomarkers in combination with egg contaminant levels, measures of reproduction, nestling thyroid hormones, and primary sex ratios. We observed significant enrichment in  $\delta^{15}\text{N}$  but not  $\delta^{34}\text{S}$  in aquatic invertebrates and dipper eggs associated with increased urbanization. Although reproductive impairment and poorer condition in nestling dippers was detected on urban impacted rivers, prey availability on impacted streams was higher than in reference areas. Nestling broods were male-dominated and plasma thyroid hormones  $\text{T}_4$  and  $\text{T}_3$  were lower in the more urbanized sites after controlling for age and size of the brood. The study revealed that observed changes in nestling dippers along urban streams impacted by sewage and stormwater runoff do not reflect the indirect effects of altered freshwater invertebrate prey but evidence suggests that direct chemical toxicity is the basis.

**RP013 Effects and Occurrence of Organic Microcontaminants in Coastal Stormwater Retention Ponds Impacted by Runoff from a Wastewater-irrigated Golf Course** L. Ferguson, Duke Univ, Dept of Civil and Environmental Engineering, Duke Univ, Nicholas School of the Environment, Pratt School of Engineering, Dept of Civil & Environmental Engineering; T. Sabo-Attwood, Univ of Florida, Dept of Environmental & Global Health; A.S. Kolok, Univ of Nebraska Medical Center, Environmental, Agricultural and Occupational Health; K. Ralston-Hooper, Visiting Dupont Research Scientist at Duke Univ, Nicholas School of the Environment; G.J. Getzinger, Duke Univ, Nicholas School of the Environment; G.A. Dominguez, Univ of Florida, Center for Environmental and Human Toxicology; L.J. Macaulay, A.J. Bone, Duke Univ, Nicholas School of the Environment. We have examined the in vivo and in vitro effects and occurrence of wastewater-derived and turf grass management organic contaminants in stormwater retention ponds impacted by sewage-derived irrigation water and located on a coastal golf course community at Kiawah Island, SC. Synthetic xenoestrogens (4-nonylphenol, bisphenol A) were detected in wastewater and retention pond water up to  $\sim 350 \text{ ng}\cdot\text{L}^{-1}$ , while biogenic estrogens (17 $\beta$ -estradiol, estrone, and estriol) were present in these samples at lower concentrations (below  $10 \text{ ng}\cdot\text{L}^{-1}$ ). Turf management chemicals such as fipronil and propiconazole were measured at concentrations  $> 1,000 \text{ ng/L}$  in stormwater retention ponds adjacent to fairway turf. In vivo estrogenic activity of stormwater and wastewater was tested by exposing male fathead minnows (*Pimephales promelas*, FHM) for 14 days to treated wastewater in an effluent holding pond as well as 3 stormwater retention ponds receiving irrigation runoff from land-application of wastewater effluent. The fish were exposed to pond water and wastewater using a novel, field-deployable, flow-through chamber system. Following the exposure, vitellogenin mRNA was quantified by qRT-PCR in livers. Additionally, to determine other biomarkers of exposure to complex mixtures containing xenoestrogens, we performed microarray and proteomic analyses on liver, gonad and brain tissues from the fish. We have also employed an in vitro largemouth bass (*Micropterus salmoides*, LMB) estrogen receptor based reporter gene assay to assess the estrogenicity of the compounds that were isolated from wastewater and stormwater ponds by solid-phase extraction and passive sampling (POCIS). For this assay, HepG2 cells were transfected with LMB estrogen receptor alpha and a luciferase reporter gene driven by 2X classical EREs. Results indicate that the effluent holding pond and one of the stormwater retention ponds induced significant estrogenicity in FHM (by qRT-PCR and microarray response) and in reporter assays. Gene expression responses were diverse among stormwater retention ponds, illustrating the complexity inherent in assessing effects of contaminant mixtures (such as stormwater) on aquatic organisms.

**RP014 Health of Fish Inhabiting an Urban Embayment** M. Gagnon, Curtin Univ, Dept of Environment and Agriculture, Curtin Univ (Bentley Campus), Dept of Environment and Agriculture, Curtin Univ, Environment and Agriculture; C. Rawson, Curtin Univ, Dept of Environment

and Agriculture; H. Nice, Dept of Water, Government of Western Australia. This study presents the results of an investigation on the health of an Australian native fish species, black bream, collected from Claisebrook Cove (Swan River, Perth, Western Australia). The sediments of this urban embayment were shown to contain the highest concentrations of polycyclic aromatic hydrocarbons (PAHs), all OC pesticides (except one) and among the highest concentrations of metals of 20 sites along the Swan River (an urban estuary in Western Australia). Physiological indices, and biomarkers of fish health were measured in black bream collected in Claisebrook Cove, and in a reference site, Burswood Lake. Black bream from Claisebrook Cove had a lower gonadosomatic index relative to those from the reference site. In addition, 15% of the males collected in Claisebrook Cove exhibited intersex, with well developed male and female gonads. Black bream captured in Claisebrook Cove had higher hepatic EROD levels indicating increased exposure to organic contaminants. This was paralleled by elevated biliary PAH metabolites. Fish captured in Claisebrook Cove had higher serum sorbitol dehydrogenase concentrations than those collected in Burswood Lake, but these were within the expected range in healthy individuals of this species. There was no difference in the oxidative DNA damage occurring in fish captured at the two sites. The results indicate that fish captured in Claisebrook Cove were exposed to a source of organic contaminants and were assimilating these contaminants (potentially via the consumption of mussels). Physiological indices indicate that the general health of fish collected in Claisebrook Cove may be compromised below that of fish in Burswood Lake.

**RP015 Effect of Urban Effluents on Fathead Minnow (*Pimephales promelas*) Gene Expression: Linkages Between Pollutants and Physiological Processes** I.A. Rodriguez, Univ of Florida, Soil and Water Science; K.J. Kroll, Univ of Florida, Physiological Sciences; G.S. Toor, Univ of Florida, Gulf Coast Research & Education Center, Gulf Coast Research & Education Center, Univ of Florida, Soil & Water Science Dept; N.D. Denslow, Univ of Florida, Dept of Physiological Sciences and Center for Environmental and Human Toxicology. In spite of all the regulatory efforts to maintain and protect habitats for wildlife, water effluents often trespass borders of wildlife refuges, potentially carrying toxic compounds. Here, we show the gene expression profiles of male fathead minnow liver, exposed to three common effluents that enter wildlife refuges in Gainesville, Florida: Surface water downstream of waste water treatment plant (streamwater), a waste water treatment plant effluent used for landscaping irrigation (wastewater), and a lake (stormwater). These waters were collected in barrels and used as whole effluents in a 48 hour exposure study under laboratory conditions. Microarray analysis was used to assess changes in gene expression for exposed male fathead minnows. After LOESS normalization and ANOVA, there were several statistically significant differences in gene expression ( $p \leq 0.05$ ) compared to controls. In the liver, 172 genes were altered for the streamwater, 349 genes for the stormwater and 327 genes for the wastewater. Enrichment analysis showed six biological processes were affected for streamwater including immune response and liver development, six for stormwater including cell cycle and transport and eight processes for wastewater including carbohydrate metabolic process and phosphate transport. Fish exposed to both streamwater and stormwater showed lipid metabolism process altered. These effluents are complex and contain many chemicals including some that behave as endocrine disruptors, for example bisphenol A and perfluorinated chemicals, among others. The microarray results suggest that these chemicals may influence normal physiological responses in exposed aquatic wildlife.

**RP016 Storm-water Treatment at Mammoth Cave National Park, Kentucky** L. Spear, Tennessee State Univ. Mammoth Cave in Central Kentucky is home to unique biological communities that have adapted to cave habitat. These organisms depend on clean water and could be harmed by contaminants carried into the cave system during storm events. Potential threats to the quality of water include vehicle petroleum leaks, improperly discarded batteries, the shoe-disinfection stations, road salts in the winter, and general parking lot runoff. This project was conducted to determine if leaf-pack filter-systems, in different stages of service, attenuated storm runoff at parking lots in Mammoth Cave National Park. The service timing of the filter systems ranged from less than 1 year, 2 years and 8 years. Grab samples were collected at the inlet and outlet of the filter systems, and analyzed for oil and grease, sediments, turbidity, gasoline compounds, nitrate, ammonia, fecal bacteria, dissolved iron, and chemical oxygen demand (COD). For the



first sampling round, the filters had not been serviced for 8 years and did very little to remove contaminants. The contaminant concentrations at the outlet were similar to those at the inlet, with the exception of removing 20-70 % of the oil and grease. Two-year old filters removed approximately 70% of the nitrate, 90% of the sulfate, 50% of the sediment and iron, but did not reduce zinc. Re-conditioned filters removed up-to 99% of the benzene, toluene, ethyl-benzene and xylene, and, up to 90% of the turbidity, *E. coli*, COD and iron from the storm runoff. Additional work is being done to determine if the filters reduce quaternary ammonia compounds; a more recent addition to the list of potential contaminants due to the installation of white-nose syndrome disinfection stations.

**RP017 Characterizing the Contaminant History of an Urban Impaired Watershed Using Lake Sediments** T.V. Willis, K.A. Wilson, Univ of

Southern Maine, Dept of Environmental Science; L.A. Benedict, Univ of Southern Maine, Chemistry; J. Staples, Univ of Southern Maine, Dept of Environmental Science. The Long Creek watershed is a 9.1 km<sup>2</sup> watershed that drains one of the most densely populated areas in the state of Maine. In the last 50 years, the watershed has undergone dramatic changes in land use and impervious cover. In the 1950s the watershed was primarily forested farmland (impervious cover < 1%) and the stream still had remnants of a historic brook trout fishery. By the 1970s, commercial development included a mall structure, small airport and interstate highway. By 2004, 38% of the watershed was in medium to high intensity development. The most heavily developed area, at the confluence of Long Creek's seven subwatersheds, is 59% impervious cover, and, as a result, Long Creek fails to meet state water quality standards. In 2009, land owners were required to take significant steps in addressing stormwater impacts based on a landmark voluntarily agreement between those landowners, MeDEP and the EPA. In this study we focus on Clarks Pond, a small impoundment located at the base of the watershed that receives runoff from all branches of the creek, and, historically, had waters sufficiently clean to supply an ice export business. We will present preliminary results characterizing metal and aromatic hydrocarbon change over time as reflected in the sediments of Clarks Pond. In winter 2011 we sampled cores from throughout the basin and sectioned the cores into 2 cm segments. Cores extended to marine clays marking the parent material underlying the impoundment sediments. Samples were dried at 50 °C and ground. Samples are currently being analyzed for metals using combustion gold amalgamation atomic absorption spectrometry (C<sub>GA</sub>-AAS) via DMA\_80 Direct Mercury Analyzer. Aromatic hydrocarbons will be assessed using a flame ionization detector (FID) after preparation of samples using microwave digestion and solid phase extraction. This study was designed to engage Univ students in meaningful environmental research and will provide a contextual framework for student-driven research and research-based curriculum that will focus on urban contaminants in the Long Creek watershed.

**RP018 Growth and Survival of Pacific Coho Salmon Smolts Exposed to Pesticides Within Urban Streams in Western Washington** K.A.

King, Washington Cooperative Fish and Wildlife Research Unit, Univ of Washington; C. Grue, Washington Cooperative Fish and Wildlife Research Unit, Univ of Washington, School of Aquatic and Fishery Sciences, Univ of Washington, School of Aquatic & Fishery Sciences; J.M. Grassley, Washington Cooperative Fish and Wildlife Research Unit, Univ of Washington; R.J. Fisk, Washington Cooperative Fish and Wildlife Research Unit, Univ of Washington, SAFS; L.L. Conquest, Univ of Washington, School of Aquatic and Fishery Sciences. We studied the growth, survival, and return rates of coho salmon (*Oncorhynchus kisutch*) smolts that had been exposed to a pesticide mixture ("cocktail") representative of that most frequently reported in urban streams in western Washington State, USA, coded-nose wire tagged, and released in spring of 2008 and 2009. Smolts were either continuously exposed from fertilization through swim-up (2007-08) or fertilization through smoltification (2007-08 and 2008-09) to pulses of the cocktail simulating that in urban streams in fall through early spring. Nominal concentrations of eight herbicides (2,4-D, dicamba, dichlobenil, MCPA, MCPP, prometon, simazine, and triclopyr), two insecticides (carbaryl and diazinon), a fungicide (pentachlorophenol), and a breakdown product (4-nitrophenol) were the either the maximum reported during fall-early spring (duration = 48 h), 20% of the maximum (72 h), or a transition between the two (48 h). With the exception of the fungicide and breakdown product, formulated products (single AI) were used, and if possible were selected from those available at retail outlets. Growth (condition factor), survival, gender, brain

AChE activity, and gonado- and hepatosomatic indices were not affected by pesticide exposure. However, return rates did differ among treatment groups and years, but overall were low (0.15-0.90%). The number of returning adults exposed to the cocktail to swim-up (0.90%, n=42) was close to twice that of unexposed controls (0.38%, n=26) in 2008, whereas in 2009, adults exposed through smoltification returned in lower numbers (0.15%, n=18) than controls (0.37%, n=30). Reasons for the apparent differences in return rates among treatments are not known, but may include differential harvest, timing of returns relative to that of hatchery operations, and effects on olfaction and immune competency. Additional studies incorporating releases of larger numbers of smolts are necessary to adequately quantify differences in return rates between pesticide-exposed and control smolts.

**RP019 Storm-water Treatment at Mammoth Cave National Park, Kentucky** A. Barzanji, Tennessee State Univ; R. Toomey, Western Kentucky

Univ & Mammoth Cave National Park; A.C. West, Tennessee State Univ, Civil & Environmental Engineering; L. Spear, Tennessee State Univ; T. Byl, US Geological Survey, Dept of Interior, Tennessee State Univ., College of Engineering. Mammoth Cave in Central Kentucky is home to unique biological communities that have adapted to cave habitat. These organisms depend on clean water and could be harmed by contaminants carried into the cave system during storm events. Potential threats to the quality of water include vehicle petroleum leaks, improperly discarded batteries, the shoe-disinfection stations, road salts in the winter, and general parking lot runoff. This project was conducted to determine if leaf-pack filter-systems, in different stages of service, attenuated storm runoff at parking lots in Mammoth Cave National Park. The service timing of the filter systems ranged from less than 1 year, 2 years and 8 years. Grab samples were collected at the inlet and outlet of the filter systems, and analyzed for oil and grease, sediments, turbidity, gasoline compounds, nitrate, ammonia, fecal bacteria, dissolved iron, and chemical oxygen demand (COD). For the first sampling round, the filters had not been serviced for 8 years and did very little to remove contaminants. The contaminant concentrations at the outlet were similar to those at the inlet, with the exception of removing 20-70 % of the oil and grease. Two-year old filters removed approximately 70% of the nitrate, 90% of the sulfate, 50% of the sediment and iron, but did not reduce zinc. Re-conditioned filters removed up-to 99% of the benzene, toluene, ethyl-benzene and xylene, and, up to 90% of the turbidity, *E. coli*, COD and iron from the storm runoff. Additional work is being done to determine if the filters reduce quaternary ammonia compounds; a more recent addition to the list of potential contaminants due to the installation of white-nose syndrome disinfection stations.

**RP020 Chloride from Roadsalt: Impacts Microbial Community Structure and the Implications for Regional Nitrogen Budgets** N. Horrell,

Univ of Connecticut, Center for Environmental Science and Engineering; J. Bushey, J. Chau, Univ of Connecticut, Dept of Environmental Engineering. Current studies indicate that shifts in microbial community structure impact their function however; this has not been extensively studied in freshwater ecosystems. As urban watersheds are impacted by increasing nitrogen and chloride loading, it is important to determine what the consequences will be for water quality and regional nitrogen budgets. Chloride levels have been shown to reach concentrations above 2 g/L during winter months due to road salt in areas with high roadway density. In urban areas nitrogen loading is usually quite high and water quality managers are under increased pressure to reduce nitrogen exports from the urban environment. In healthy streams, only 10-25% of the nitrogen from the terrestrial landscape is exported to the coastal ecosystem which is a testament to the value of the ecosystem service provided by these streams. The microbial community is a key player in the nitrogen cycle and if the health of this community is degraded, water quality will also be degraded. This summer I will be collecting sediment samples to analyze microbial community structure and function. First I will collect sediment samples and incubate them with varying concentrations of chloride. Terminal Restriction Fragment Length Polymorphism (TRFLP) analysis will be conducted prior to and after the incubations to get an approximation of the shift in microbial community structure. After we confirm that there is a shift in microbial community structure in the sediments that are collected, we will perform similar incubations with the addition of nitrogen as nitrate and determine denitrification rates. Additionally water samples will be measured at the end of the incubation to verify the amount of nitrate that utilized for denitrification. The end result will be data that shows the shift in microbial community structure as a function of chloride and how

this impacts nitrogen utilization in the sediments. Our hypothesis is that the diversity of microbial population will decrease as we increase the chloride concentration and that this will also decrease the rates at which nitrogen is removed from the water column through denitrification. This will provide water quality managers important insight into how increasing chloride concentrations will impact regional nitrogen budgets.

**RP021 Estimating Impacts on Coho Salmon Populations from Land Use-related Spawner Mortality in Urban Streams** J. Spromberg, NOAA Fisheries, Northwest Fisheries Science Center, NOAA Fisheries, Environmental Conservation Dep; D. Baldwin, B. Feist, E. Buhle, N. Scholz, NOAA Fisheries. Since the late 1990s, monitoring efforts evaluating the effectiveness of urban stream restoration projects in the greater metropolitan area of Seattle, Washington, have detected high rates of premature mortality among adult coho salmon (*Oncorhynchus kisutch*) in restored spawning habitats. Affected animals display a consistent suite of symptoms (e.g., disorientation, lethargy, loss of equilibrium, gaping, fin splaying) that ultimately progresses to death on a timescale of a few hours. Annual rates of pre-spawn mortality (PSM) observed over multiple years across several drainages have ranged from ~20% to 90% of the total fall run within a given watershed. The current understanding of coho PSM is that it occurs when pollutants accumulate on impervious surfaces during summer and early fall dry periods are then washed into coho-bearing streams by fall storm events. The phenomenon seems to require both specific land uses and precipitation patterns to occur. To evaluate the relationships between PSM, land use and precipitation patterns, we ran a series of spatial analyses to detect correlations between land cover (roadways, impervious surfaces, forests, etc.) patterns, seasonal rainfall patterns and PSM rates in 6 watersheds. The relative proportion of local roads, impervious surfaces, and commercial property in the catchment was most strongly correlated with coho PSM rates. Further, our analyses suggest that as urban expansion continues, areas that once supported coho salmon may experience PSM rates that could jeopardize the coho salmon population viability. So, to evaluate the potential consequences of current and future urbanization on wild coho salmon, we constructed life-history models to estimate the impacts of pre-spawn mortality on coho populations and metapopulations. At the low (20%) and high (90%) ends of the range of observed mortality, model results indicated the mean time to extinction of localized coho populations in 115 and eight years, respectively. The presence of productive source populations (i.e., unaffected by pre-spawn mortality) within a metapopulation reduced local extinction risk. However, as more populations within a metapopulation become affected by spawner die-offs, the source population's productivity declined. These simple models demonstrate the potential for rapid losses from coho populations in urbanizing watersheds.

**RP022 Effects of Road Salt on Hydraulic Conductivity in Stormwater Retention Ponds, Owings Mills, MD** R. Casey, Towson Univ, Urban Environmental Biogeochemistry Laboratory, Towson Univ, Dept of Chemistry; M. Gallagher, Lawrence Univ, Dept of Geology; K. Linford, J. New, S.M. Lev, Towson Univ, Urban Environmental Biogeochemistry Laboratory. The abundance of impervious cover in urban areas impacts stream geomorphology and in turn local hydrology by directing stormwater runoff into streams. In Maryland, mitigation of these impacts has led to the installation of stormwater retention ponds. In colder climates, these ponds also receive deicing salts which are applied during winter storm events. Sodium chloride (NaCl) is the most commonly used salt for this purpose. It is possible for this salt to impact the structure of pond soils if present in high enough concentrations. Soil structure collapse can occur as sodium replaces other cations bound to the surface of soil clay particles. An automated mini-disk infiltrometer (AMD1) array was used to measure infiltration rate at two sites within seven different ponds in the Red Run watershed, Owings Mills, MD. The hydraulic conductivities (max. = 4) at each location were then averaged to determine a composite hydraulic conductivity for the test site. In order to calculate an accurate hydraulic conductivity the test soil needed to be classified according to the USDA soil classification scheme. Composite soil samples from each site were sieved using 5 mm, 2 mm, 63µm dry sieves and a 5 µm wet sieve. The mass percentage of each fraction smaller than 5 mm was used to determine the USDA soil classification. Each soil sample was also analyzed using ion chromatography to determine the concentration of major cations present. Soils from stormwater retention ponds in the Red Run watershed exhibited a large range in sodium concentrations due to the application of road salt and there was a significant negative relationship

between sodium concentration and hydraulic conductivity. The areas within these ponds that are regularly inundated tended to have higher soil sodium concentrations and significantly lower hydraulic conductivities resulting in a retarded infiltration rate. Because infiltration is being retarded, these ponds may not be adequately performing the functions for which they were designed, potentially resulting in increased loading of stormwater and associated contaminants into adjacent surface receiving waters.

**RP023 Assessment of Urban Road Dust in Tokyo Using Whole Sediment Toxicity Identification Evaluation Procedures** H. Watanabe, National Institute for Environmental Studies, Center for Environmental Risk Research; F. Nakajima, The Univ of Tokyo, Environmental Science Center; N. Tatarazako, National Institute for Environmental Studies, Center for Environmental Risk Research, National Institute for Environmental Studies, Endocrine Disrupter Research Laboratory; I. Kasuga, The Univ of Tokyo, Dept of Urban Engineering; H. Furumai, The Univ of Tokyo, Research Center for Water Environment Technology. Road dust is considered as an important source of sediment contamination; however, there are few studies on the toxicity of road dust on benthic organisms. This study evaluated the toxicity of road dust to the benthic ostracod, *Heterocypris incongruens*, through a 6-day direct exposure test. Moreover, in order to identify the main groups of toxicants in road dust, we applied the whole sediment toxicity identification evaluation (TIE) methods to road dust in Tokyo. Six road dust samples (St.1-6) collected using a vacuum cleaner from street gutter in heavy traffic areas and from highway parking area caused high mortality of the ostracod, whereas the road dust collected in a residential area (St.7) did not show toxicity to the ostracod. Road dust collected using a street sweeper truck from a highway (St.8) also did not show lethal toxicity. Three out of six toxic samples (St.1-3) were subjected to the TIE experiment. The addition of Ambersorb and XAD completely eliminated the toxicity in all the samples. It is suspected that hydrophobic compounds were main toxicants in the road dust samples. In addition, Chelex treatment also resulted in a considerable reduction of the mortality to 3-10% in two samples (St.2, 3). However, the measured concentrations of dissolved Cd, Cu, Ni, Pb and Zn in the overlying water of the toxicity tests did not exceed the LC50 values in literature. To test a hypothesis that the toxicity reduction by XAD and Chelex in St.3 sample was due to the removal of hydrophobic compounds, not heavy metals, a toxicity test was conducted on the fractions eluted with organic solvents from XAD and Chelex which were recovered from the TIE experiment. Methanol-extracted fractions of both the recovered adsorbents, XAD and Chelex, showed 100% mortality of the ostracod, indicating that the hydrophobic organic compounds removed by XAD and Chelex were the principal toxicants in the road dust from St.3.

**RP024 Decision-making Framework for Identifying and Communicating Benefits and Risks of Disinfecting Wet-weather Flows** P.E. Goodrum, Environmental Resource Management; J. LaGorga, Stearns & Wheeler GHD; S.E. Boehme, ERM, Sediments and Watershed Management. Most wet weather flows contain high levels of bacteria that may pose health risks to recreational users and aquatic organisms in receiving waters. Water quality impacts of combined sewer overflows, sanitary overflows, and stormwater discharges are highly site-specific, temporally variable, and sporadic in occurrence. Chlorine has long been the most common disinfectant for drinking water, wastewater, and wet weather flows. Literature suggests that treatment technologies can decrease concentrations in effluent of some compounds, including pharmaceuticals, but may have little treatment impact on others. In addition, disinfectants added to wet weather flows can result in residuals and react with organic matter in the water to form disinfection byproducts (DBPs). The types and quantity of DBPs is a function of the disinfection type and dose, the length of contact time, and the water quality characteristics inherent to the wet weather flow. Concerns regarding chlorine disinfection are mainly in two areas: (1) safety in handling and use, and (2) potential impacts of residuals and DBPs on human health and the aquatic ecosystem. Viable alternatives to chlorination/dechlorination treatment technologies include chlorine dioxide, ozonation, and ultraviolet (UV) irradiation. A decision-making approach was developed and implemented in a demonstration project in Onondaga County to select the most sustainable wet weather flow disinfection technology that balances benefits and risks, including social and economic impacts. This framework was then considered for application to full-scale facilities to determine the key site-specific factors that have the greatest influence on the selection of an appropriate treatment technology. Results from the pilot study may be used to guide future studies

to evaluate byproducts associated with treatment alternatives applied to a broader class of chemicals, including pharmaceuticals.

**RP025 Effects of Salinity Derived from Road Salt and Total Suspended Solids on Yellow Perch Eggs and Larvae from Two Chesapeake Bay Tributaries** G. Ziegler, Univ of Maryland, Wye Research & Education Center; A. Pinkney, US Fish and Wildlife Service, Chesapeake Bay Field Office; D. Ownby, Towson Univ, Urban Environmental Biogeochemistry Laboratory; D.J. Fisher, Univ of Maryland, Wye Research & Education Center; R. Casey, Towson Univ, Urban Environmental Biogeochemistry Laboratory, Towson Univ, Dept of Chemistry. Yellow perch (*Perca flavescens*) have historically been a major commercial and recreational fishery in the Chesapeake Bay watershed. A semi-anadromous species, it lives in fresh to brackish waters of many Chesapeake Bay tributaries, and migrates to upstream freshwater habitats to spawn. The primary movements are the upstream spawning migration of adults and the downstream dispersal of juveniles. Although Bay tributaries like the Choptank have healthy populations of yellow perch, with no indication of egg/larval viability problems, Severn River populations are doing poorly with evidence of depressed egg and larval viability. Two of the potential causes are elevated salinity and total suspended solids (TSS). The goal of this study is to analyze the impacts of salinity (ambient estuarine and road salt adjusted well water) and exposure to suspended sediments on the survival of yellow perch eggs and larvae from two rivers, the Severn and the Choptank. Adult yellow perch were collected and spawned in a hatchery and fertilized eggs were transported to the test facility. Year one of this study looked at the effect of salinity, while year two studied the combined effect of salinity and TSS, since little is known about the potential for synergistic effects. Eggs and larvae from the Severn and Choptank Rivers were maintained at three salinities (1.5, 3 and 6‰ derived from diluted estuarine water or road salt in well water), Severn River water, or well water as a control. Eggs were tested till hatch out (approximately 2 weeks) while larvae were tested for 5-7 days. Water samples were analyzed for dissolved metals, cations, and anions. Percent hatch, time to hatch, survival and abnormality data was collected from eggs, while survival and abnormality data was analyzed for larvae. No effects were observed for either Choptank or Severn larvae when exposed to elevated salinities. Although total hatch out of eggs was unaffected, the time to hatch and number of malformations increased in eggs from both rivers in 6‰ road salt; and for Severn River eggs only in 6‰ estuarine water. Interestingly, time to hatch was also increased for both egg types exposed to Severn River water samples at 1‰. Results from year one salinity tests and year two, combined effects of TSS and elevated salinity, will be presented.

**RP026 Runoff of Fipronil from Concrete Surfaces Following Simulated and Natural Rainfalls** W. Jiang, Univ of California, Riverside, Dept of Environmental Sciences, Univ of California, Riverside, graduate student; D. Haver, Univ of California South Coast Research and Extension Center; A. Soeprono, M. Rust, Univ of California, Riverside, Dept of Entomology; J. Gan, Univ of California, Riverside, Dept of Environmental Sciences. Increasing fipronil use for urban pest control has resulted in their occurrence in urban runoff and contamination of urban streams and estuaries. In this study, we investigated the runoff behaviors of fipronil following simulated and natural rainfalls from concrete surfaces, and evaluated influences of precipitation intensities and concrete surface conditions on fipronil runoff transferability. High levels of fipronil were found in the runoff 1 d after pesticide treatment, and the concentrations of fipronil in the runoff water rapidly decreased as the pesticide residence time on concrete increased. Fipronil exhibited long persistence on the concrete and prolonged runoff transferability. After 3 months under summer conditions and four wash-offs, fipronil residues were still detected in the runoff water at  $0.18 \pm 0.06$  ppb. Fipronil residues were detected in the runoff from natural rainfalls even 7 months after the initial pesticide application, implying that fipronil applied early in the year may still be available to contaminate winter storm runoff. Precipitation intensities and modifications to concrete surfaces did not significantly influence fipronil runoff behaviors. A surface wiping method was developed to measure fipronil residues on the concrete and the results showed good correlations between the wiping-extractable residues and fipronil concentrations in the runoff water.

**RP027 Occurrence of Fipronil Degradates in the Runoff from Concrete Surfaces** W. Jiang, Univ of California, Riverside, Dept of Environmental Sciences, Univ of California, Riverside, graduate student; D. Haver,

Univ of California South Coast Research and Extension Center; M. Rust, Univ of California, Riverside, Dept of Entomology; K. Goh, California Dept of Pesticide Regulation; J. Gan, Univ of California, Riverside, Dept of Environmental Sciences. Due to the increasing use of fipronil in urban areas for termite and ant controls, fipronil degradates have been widely detected in urban streams throughout the United States. Fipronil metabolites exhibit similar or even higher toxicity to non-target aquatic organisms than fipronil itself, but the causes and pathways for fipronil and its metabolites to contaminate urban surface waters are not well understood. In this study, concrete surfaces were treated with a commercial formulation of fipronil, and the occurrence of three major fipronil degradates, i.e., desulfinyl fipronil, fipronil sulfide, fipronil sulfone, in the runoff water from the treated concrete surfaces were evaluated following simulated and natural rainfalls. High concentrations of fipronil degradates were found in the runoff water 1 d after the treatment, and these degradates were also detected in runoff water after 3-month exposure under outdoor summer conditions. The fipronil degradates were further found in the runoff from the first two natural winter rainfalls after 7 months from the fipronil treatment. Over 80% of fipronil degradates in the runoff water were in the dissolved aqueous phase. The results indicate that use of fipronil around residential homes may serve as an important source for sustained contamination of fipronil metabolites to urban surface streams.

**RP028 Assessment Priorities for Emerging Contaminants in the Delaware Estuary** R. MacGillivray, Delaware River Basin Commission, Dept of Modeling, Monitoring and Assessment. Emerging contaminants are unregulated substances that have entered the environment through human activities. Current regulatory approaches are inadequate to address these contaminants and the increasing public concern over their environmental and human health implications. Six sites in the tidal Delaware River were sampled in 2007, 2008 and 2009 for emerging contaminants. Water samples collected during these surveys were analyzed for 119 pharmaceuticals and personal care products, 13 perfluorinated compounds, 17 hormones, 3 nonylphenols and bis-phenol A. Concentrations of emerging contaminants in the Delaware Estuary were compared to concentrations found in surface water at other locations and to benchmarks developed for environmental health and safety by other regulatory agencies. Assessment priorities in the estuary are identified including further characterization of persistent and bioaccumulative perfluorinated compounds and a more comprehensive evaluation of potential ecological effects from pharmaceuticals.

**RP029 Prioritizing Chemicals Used in PCPs in China for Environmental Risk Assessment: Application of the RAIDAR Model** T. Gouin, Unilever, Safety and Environmental Assurance Centre; R. van Egmond, O. Price, Unilever; J. Mortimer, Unilever, Unilever, SEAC. Brazil, Russia, India, and China (BRIC) are expected to be the four biggest economies by 2050. Of these four countries, China is commonly recognized as the biggest potential market for home and personal care products. It is thus important that infrastructure and regulatory instruments are sufficient to reduce environmental impacts related to economic growth and use of such products. Chemicals used in personal care products (PCPs) represent a significant fraction of chemicals used in commerce in China. The environmental fate and effect datasets of many cosmetic ingredients, however, are limited. Recently, there has been emerging concern regarding the use of a number of substances classified under this category, including the nitro- and polycyclic musks, UV blockers such as methylbenzylidene camphor, and preservatives such as the parabens. Given the continuous emission of chemicals used in PCPs to wastewater and the aquatic environment after regular use during showering or bathing, methods for prioritising the environmental risk assessment are needed. In an effort to address this knowledge gap we have identified the chemical ingredients used in 2500 PCPs across China, and estimated the annual emission of these chemicals. The physical-chemical property data for these substances have been estimated and used as model inputs in the Risk Identification And Ranking (RAIDAR) model. A sensitivity analysis relating to model input parameters and environmental release scenarios is performed as a method for assessing the influence of input parameters with respect to chemical ranking and prioritisation.

**RP030 Probabilistic Evaluation of Mammalian Pharmacology Data to Target Human Pharmaceuticals for Environmental Hazard Assessment** J. Berninger, Baylor Univ, Institute of Biomedical Studies; B.W. Brooks, Baylor Univ, Environmental Science and Biomedical Studies, Baylor Univ,



Dept of Environmental Science. It is well established that human pharmaceuticals are present in the water, sediment, bio-solids, and biota of aquatic systems. Which drugs are present and what effects they may have on aquatic organisms is less well understood. Because hundreds of drugs enter aquatic systems, the need for prioritization of research efforts is evident. To this end, several different prioritization schemes have been postulated, though each has limitations. For example, our previous research has focused on leveraging mammalian pharmacological potency data to potentially identify chemicals for future study, but there are many other potentially useful pharmacokinetic and pharmacodynamic parameters. The objective of this study is to evaluate an extensive series of mammalian pharmacological parameters for 600 drugs using a probabilistic model, which is similar to approaches used in development of species sensitivity and chemical toxicity distributions. Using this approach we identify the probability of encountering therapeutic parameters in general and within specific classes in particular for margin of safety,  $C_{max}$ , acute toxicity ( $LD_{50}$ ), volume of distribution, clearance rate, half life,  $\log P$ ,  $\log D$ ,  $pK_a$ , and BCF. For example, with  $C_{max}$  there is a 10% probability of encountering a value below  $0.002 \mu\text{g/mL}$ ; while for volume of distribution there is a 10% probability the value will be greater than  $10 \text{ L/kg}$ . We demonstrate how various centiles of important parameters may be used for ranking compounds for future study. Further, the potential relevance for integration of this approach within various prioritization frameworks is discussed.

**RP031 Use of QICARs to Predict the Speciation of Data-poor Metals and Their Toxicity Towards Aquatic Organisms** S. Le Faucheur,

INRS – Eau, Terre et Environnement; C. Fortin, INRS – Eau, Terre et Environnement, Université du Québec; P.G. Campbell, INRS-Eau, Terre et Environnement, INRS-ETE, INRS, Centre Eau Terre Environnement. To deal with less common metal-containing compounds, governmental agencies will require information about the speciation and inherent toxicity of many “data-poor” elements. In this context, we explored the possible use of quantitative ion character-relationships (QICARs) to estimate the speciation and the relative toxicity of some rarely studied elements in model natural waters. The studied elements were bismuth, the Platinum Group Elements (PGE) and the lanthanides. The prediction of the speciation of the studied elements in natural freshwaters was initiated with a screening of the available geochemical databases, gathering complexation constants between metals and dominant inorganic ligands. Chemical equilibrium models (here VMINTEQ) were then used to calculate the inorganic speciation of the elements in two typical Canadian surface waters in the presence and absence of natural organic matter. Stability constants for Bi and PGE are scarce relative to those for the lanthanides. The simulations showed that the contribution of the free lanthanide ions to the solution speciation should be low in the presence of natural organic matter, suggesting that their bioavailability should also be low under these conditions. Among predictive models for metal toxicity found in the environmental toxicology literature, we selected two promising models to predict the toxicity of our data-poor elements. The first model was published by Wolterbeek and Verburg (Sci. Tot. Environ. 279: 87-115 (2001)). This model successfully relates the electrochemical and ionization potentials, electronegativity, atomic radius and atomic weight of a given metal to its toxicity towards a variety of aquatic organisms. The second model was published by Kinraide (Environ. Toxicol. Chem. 28: 525-33 (2009)) and uses a “consensus scale of softness” and a “consensus scale of toxicity” to predict the toxicity of 92 ions. Both models predict that the elements of the PGE as well as Bi should be more toxic than the elements of the lanthanide group. Moreover, Bi, Ir, Pt and Pd are predicted to have comparable or higher toxicities than the more “common” metals (e.g., Cd, Hg and Pb). Information provided by the present study should be useful for designing new toxicity tests for the little-studied elements and also for determining priorities based on which metals are likely to be the most problematic.

**RP032 Occurrence of Pharmaceuticals and Steroids in Sewage Effluent and Surface Waters of the Rio de la Plata Estuary and Pampa's Shallow Lakes, Argentina** Y. Elorriaga, D.J. Marino, Centro de Investigaciones del Medio Ambiente – UNLP, CONICET, Dto. Química, Facultad de Ciencias Exactas; P. Carriquiriborde, Conicet, Centro de Investigaciones del Medio Ambiente, Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Química, Centro de Investigaciones del Medio Ambiente – UNLP, CONICET, Facultad de Ciencias Exactas, UNLP; A.E. Ronco, Centro de Investigaciones del Medio Ambiente – UNLP, CONICET, Dto. Química,

Facultad de Ciencias Exactas. Over the last years emerging pollutants have gained the attention of the public and scientific community. However, the occurrence of this group of contaminants in some parts of the world is still almost unknown. In the present study, a group of pharmaceuticals together with synthetic and natural steroids were analyzed in sewage effluent and surface water samples collected from urbanized sites on southern coast of the Rio de la Plata Estuary and Pampa's Region shallow lakes. A total of 14 pharmaceutical and four steroids were analyzed. They were selected on the bases of a survey of use. Sampling sites were selected according with the sewage effluent outfall location, collecting the water samples at increasing distances from the discharge. Samples were filtered through  $0.45 \mu\text{m}$ , extracted by SPE, and then analyzed using a HPLC system (Agilent 1100) with either a C18 (steroids) or a PFP (pharmaceuticals) column and attached to a MS (G1956A MSD VL) equipped with both ESI (pharmaceuticals) and APCI (steroids) sources. In the effluent samples the following pharmaceuticals were detected: caffeine, paracetamol, atenolol, carbamazepine, ciprofloxacin, ibuprofen, diclofenac, loperamide, clonazepam. The same group of compounds, but at lower concentrations, was detected in the surface waters samples near the discharge. At 1 km downstream the discharge caffeine, carbamazepine, ibuprofen and diclofenac were still detected. Mayor identified steroid in the sewage and surface water samples were estrone, ethynilestradiol, and estradiol, presenting concentrations concentration between  $300\text{--}100 \text{ ng/L}$  in the effluents and  $25\text{--}4 \text{ ng/L}$  in the surface waters collected next to the discharges. The study confirmed previous concentrations of steroids found in sewage and surface waters of the Rio de la Plata Estuary and Pampa's shallow lakes, and reported for the first time the occurrence of pharmaceuticals for this region of Argentina.

**RP033 Evaluating the Impact of Dispersed Oil from the MC252 Deepwater Horizon Incident Based on Laboratory Studies of Field-collected Water and Sediment** M. BenKinney, Exponent; W. Bryant, US Geological Survey; M. Edwards, Exponent; J. Biedenbach, US Geological Survey; J.S. Brown, Exponent. As part of the sub-sea and sub-surface sampling program to determine the location, fate, transport and threat of oil and dispersant from the MC252 Deepwater Horizon incident, an investigation of the coastal nearshore water and sediment was initiated on behalf of the Unified Area Command (UAC) in the Gulf of Mexico by multiple parties, including the US Environmental Protection Agency (EPA), BP, and the US Geological Survey (USGS). Samples were collected along the coastline using water column fluorometry profiles, water quality measurements, and collection of sediment and water for chemical analyses and toxicity studies to assess the environmental fate of the dispersed Macondo oil. Fluorometry casts were used as an operational field tool in deeper coastal water to measure fluorescence as an indicator of polycyclic aromatic hydrocarbons (PAH) in the water column at each station. Water quality measurements of particle size, conductivity, temperature, pH, and dissolved oxygen were measured at depth intervals at each station. Chemical analysis and toxicity testing were performed on water samples collected at depth and on sediment grabs (top 2 cm of the grab sample) collected by hand or using a grab sampler (e.g., Ponar, modified double VanVeen). Samples were analyzed for hydrocarbons (benzene, toluene, ethylbenzene, xylenes [BTEX]; total petroleum hydrocarbons [TPH] and saturated hydrocarbons; PAHs; petroleum biomarkers in sediment (dipropylene glycol n-butyl ether [DPnB], propylene glycol, and dioctylsulfosuccinate [DOSS]), and sediment physicochemical characteristics (total organic carbon [TOC] and grain size). Toxicity tests were conducted in the laboratory with representative fish, marine shrimp, sea urchins, amphipods, worms, molluscs, diatoms, and algae. Limited effects outside the range of acceptable natural variability were seen in all species, with the amphipod showing greater sensitivity than the in-water species.

**RP034 Hebei Spirit Oil Spill in Korea: Temporal Trends of Petroleum Hydrocarbons in Oyster (*Crassostrea gigas*)** S. Ha, U. Yim, M. Kim, G. Han, J. An, S. Hong, Korea Ocean Research and Development Institute, Oil and POPs research group; J. Jung, Korea Ocean Research and Development Institute; W. Shim, Korea Ocean Research and Development Institute, Oil and POPs research group. An oil tanker named *Hebei Spirit*, spilled an estimated 12,547 kL of crude oil of three kinds, at the entrance of Taean coast, Korea on December 7, 2007. Temporal variation in petroleum-derived hydrocarbons in oyster (*Crassostrea gigas*) was investigated at Taean coastal region after the *Hebei Spirit* oil spill (HSOS). Oil contamination in oyster, in the survey area, during December 2007, rose to 10-1000 times over its pre-spill level. The PAHs concentration increased significantly after the

spill and decreased 7-8 months later close to the background level. Among the PAH homologues, alkyl-substituted dibenzothiophenes were highest in the relative proportion, except naphthalenes, and followed by alkylated phenanthrenes. This compositional signature in oyster is consistent with those of spilled oil. PAHs composition profile and chemical fingerprints in oyster revealed that the impact of spilled oil continued until the next spawning season. PAHs concentrations in oyster showed seasonal variations at all stations thereafter. Since oyster does not metabolize petroleum hydrocarbons significantly, the compositional profile in oyster can be effectively used to trace the source of oil pollution in the marine environment.

**RP035 Use of Principal Component Analysis and Source Apportionment for PCBs in Sediment: An Urban Bay Example** E.L. Butler, Gradient; C.B. Tuit, Gradient, Associate; E. Wannamaker, Gradient. Polychlorinated Biphenyls (PCBs) are persistent organic pollutants that become associated with particles upon discharge, and are often a major concern in urban waterways because of their concentrations in sediments relative to their toxicity. PCBs had many uses in commerce and there were frequently many sources of PCBs in urban sedimentary environments. This talk will describe the use of various lines of evidence to identify a distinct source of PCBs to an urban bay. A number of different data sets pertaining to bay sediments were evaluated and combined for analysis. These data provided PCB congener and PCB homolog results, but not Aroclor results. A spatial analysis of the data revealed the likely presence of a distinct source of PCBs. Principal Component Analysis (PCA) was performed on the PCB homolog data to elucidate relationships among the samples and the similarity of their PCB distributions to various Aroclors. The PCA corroborated the spatial analysis and identified the Aroclors that were involved. This step was important because consumers purchased and used Aroclors, not PCB homologs or PCB congeners *per se*. Subsequently, an Alternating Least Squares (ALS) apportionment method was used to estimate the amount of each Aroclor that comprised the PCBs in each sediment sample. Mapping of the results, along with aerial photography showing the location of a large industrial discharge leading into the bay, provided strong evidence of a previously unidentified contributor of PCBs to sediments in the bay.

**RP036 Toxicity Tests as Primary Tool for Assessing Sediment Quality** W. Ahlf, TU Hamburg-Harburg, Environmental Science and Technology Dept, TU Hamburg-Harburg, Environmental Technology and Energy Economics. Toxicity tests are recommended in concepts for the assessment of contaminated sediments. Due to the heterogeneity of the matrix (sediment) and the genetic variability of test organisms, toxicity tests are often undervalued in relation to their statistical power. In most cases standardized tests are performed with the same requirements for quality control as chemical analyses have. Consequently, toxicity testing can only be done with a limited number of species, tested under controlled laboratory conditions, thus limiting their ecological relevance. Quality Assurance/Quality Control (QA/QC) requirements for the biological tests are highly specific, because a calibration using reference material does not cover all aspects of biological variability. Another crucial point is the quantitative comparison of toxic effects. Sensitivity of toxicity tests is not only species and contaminant-specific; it also varies depending on the endpoint measured. The variation and standard deviation of each bioassay has to be known in order to categorize toxicity reliable. Information on the responsiveness of a test system is hence of high importance for the interpretation. In this lecture we suggest an approach on how to set up a site-independent classification system for sediment toxicity tests, emphasizing the following steps: the assessment of test-specific response spans for each applied biotest and the subsequent interpretation of inhibition values in terms of toxicity, estimation of accuracy and uncertainty of different test systems. A brief overview will be given how control procedures ensure the quality of ecotoxicological tests. Finally a bioavailability concept will be presented to reduce uncertainty for the derivation of sediment quality standards and enhance ecological relevance, as requested for the European regulation.

**RP037 The Application of Systems-based Analysis and RNA-Seq Technologies in Ecotoxicology** F. Brinkman, Simon Fraser Univ, Dept of Molecular Biology and Biochemistry; L.L. Brown, Univ of Simon Fraser, Molecular Biology and Biochemistry, Environment Canada, Environmental Toxicogenomics. In the last decade, there has been a surge of transcriptomic data generated in the field of ecotoxicogenomics due to the availability and rising popularity of tools such as microarrays, qPCR and most recently,

RNA-Seq. Analyses often result in large gene lists of up/down regulated genes that, alone, may not be very insightful. Microarrays, in particular, are fraught with noise that can hide more subtle, but still significant, gene expression changes. Using a systems-based approach, one can examine transcriptomic profiles in a more pathway-based context, or larger molecular interaction network-based framework – essentially treating the organism as the whole system that it is. This approach becomes even more powerful when coupled with RNA-seq, which has better signal-to-noise and higher dynamic range than microarrays. We present analyses of transcriptomic data in this context, data generated by ourselves and re-analyses of others, illustrating both the potential and challenges of this approach.

**RP038 The Importance of Normalization on Large and Heterogeneous Microarray Datasets** E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, Environmental Processes and Effect Division; T. Habib, Badger Technical Services; L. Burgoon, US Environmental Protection Agency, National Center for Environmental Assessment; S. Edwards, US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory; F. Falciani, School of Biosciences, Univ of Birmingham; A. Loguinov, Univ of California at Berkeley; D. Villeneuve, USEPA National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; C. Vulpe, Univ of California at Berkeley; R. Taylor, Pacific Northwest National Laboratory, US Department of Energy; N. Garcia-Reyero, Jackson State Univ. DNA microarray technology is a powerful functional genomics tool increasingly used for investigating global gene expression in environmental studies. Microarrays can also be used in identifying biological networks, as they give insight on the complex gene-to-gene interactions, networks and pathways, thereby enabling the exploration and examination of how chemicals cause toxicity. Gene expression analysis is a multi-step process and there are many sources contribute to systematic variations that can affect the measured gene expression levels. Normalization is one step to minimize the systematic variations in the measured gene expression levels. Appropriate normalization procedures must be implemented so that the expression levels can be effectively compared across biological samples within an experiment and between different experiments. In this study we used the dataset composed of 1,472 single color 15k Agilent arrays from different experiments. All experiments were performed in the same laboratory, on the same tissue (fathead minnow ovary), and with a range of treatments for which we can hypothesize the original assumptions to be correct. Non-linear normalization methods: quantile normalization and a slightly modified cyclic loess normalization, fastlo normalization. We applied two network inference algorithms, based on mutual information, Accurate Cellular Networks (ARACNE) and Context Likelihood of Relatedness (CLR) to infer the network model from combining the datasets. Results indicated that fastlo was found to be best normalization as the correlations between interacting genes were enhanced and models obtained from combined datasets revealed that the networks were associated with specific biological processes or potential relevance for ovary biology.

**RP039 The Sampling Protocol and Measurement of Trace Organics: Recent Learnings** K.V. Ohman, Associated Engineering Alberta Limited; M. Chen, City of Calgary, UEP – Water Resources; D. Smith, Univ of Alberta, Civil Engineering; S. Smyth, Environment Canada. Over the last fifteen to twenty years in Europe, North America, and elsewhere, there has been growing interest and concern about the potential impact of trace organics on the health of the local and regional environment. A number of jurisdictions in North America and Europe have undertaken studies to determine the concentrations, and quantity of these constituents, and the ability of water and wastewater systems to remove these compounds, as well as their impact on human health and the local ecosystem. What is often overlooked and poorly understood, is the sampling and analytical tools used to measure these parameters and the subsequent level of uncertainty that must be applied to the results used by water and wastewater facility design and operations personnel. As with many analytical parameters the location, type, and timing of sample collection is critical to the value derived from the sample analysis. There are concerns related to the type of sample container used which impact sample integrity, degradation, adsorption, volatilization, photo-degradation, as well as other physical actions that may impact each trace organic differently. Sample handling, storage temperature (i.e., freezing) and holding time exceedances can have a significant impact on transformation mechanisms that may occur. The container material and surface to volume

ratio can influence concentration levels in the final result. Furthermore, the analytical methods matrix effect, the holding time, uncertainty level and recovery rate associated with the particular instrument and method, and analyst will have a significant impact on the final result. Furthermore, the level of data validation and proficiency testing (both of which seldom apply to analytical tests undertaken for these compounds) need to be considered when using the published laboratory data. The practitioner needs to factor in these aspects when evaluating the analytical results. Mistaken conclusions can ultimately cost utility owners and operators millions of dollars which in turn can undermine the credibility of their staff and the associated customers or clients. This paper addresses the stewardship of trace organic compounds from the collection source to the production of the analytical results. It also addresses the level of diligence required to ensure a result that in spite of the best of intentions and a high level of technical ability, may still carry with it, a very high degree of uncertainty.

**RP040 The Use of a New Class of Sewage Tracers to Characterize the Sources and Fate of Di-ethylhexyl Phthalate in a Large Sewage Affected Coastal Lagoon** A.C. Doherty, Stony Brook Univ, School of Marine and Atmospheric Sciences, Stony Brook Univ, graduate student; B.J. Brownawell, Stony Brook Univ, School of Marine and Atmospheric Sciences.

There are generally multiple point and non-point sources of chemical contaminants to urban or highly developed estuarine and coastal environments. Recent research indicates that a group of hydrophobic organic cations, quaternary ammonium compounds (QACs), can serve as stable, particle reactive sewage tracers to separate the sources and differential fate of other strongly sorbed contaminants, yet application in environments with a single sewage source have been lacking. Hempstead Bay is located on the southwest shore of Long Island, NY and receives 70 million gallons per day of sewage into a restricted area. A study has been undertaken to distinguish sources of the particle reactive di-ethylhexyl phthalate (DEHP), a known marine contaminant and suspected endocrine disrupting compound, within the Bay. DEHP sediment concentrations varied from an average value of 2.6 µg/g close to the sewage source and decreased steadily with distance away (over 20 km). QAC sediment concentrations were as high as 104 µg/g and were linearly related to DEHP ( $r^2=0.94$ ) with a zero intercept, suggesting sewage as a dominant source of DEHP in this environment. Results also suggest that particle-bound DEHP is persistent in Hempstead Bay. Additional work will be presented that examines the relationship between QACs, DEHP and trace metals in areas where other sources may be more important. While sewage is known to be a potentially important input of phthalate esters, this is the first work that suggests that it can be the dominant source of DEHP in coastal marine waters.

**RP041 Short-term Weathering Profile of Oil Released from the Deepwater Horizon Blowout from Surface and Shallow Subsurface Samples** A. Revill, D. Fuentes, S. Armand, A. Ross, X. Qi, E. Crooke, C. Stalvies, A. Talukder, CSIRO. The Deepwater Horizon incident began on April 20th 2010 and oil was released into the Gulf of Mexico until July 15th 2010.

Although there is evidence for some entrainment of oil at depth, significant quantities of the oil reached the surface. Different components of crude oils are known to be subjected to evaporation, dissolution, photo-oxidation and biodegradation at different rates. Analysis of specific components provides the potential to assess the degree to which individual samples have undergone each of these processes. Between June 5<sup>th</sup> and 15<sup>th</sup> September 2010, 440 surface seawater and slick samples were collected in the Northern Gulf of Mexico. A reference sample of oil recovered from the Macondo well was available for calibration purposes in the field. The samples were subjected to liquid-liquid extraction followed by gas chromatography mass spectroscopy onboard the vessel. This work was in support of the calibration efforts of the CSIRO hydrocarbon sensor survey which collected 8,998 linear nautical miles (~16,664 line kilometers) of fluorimeter sensor data, gathering over 4.5 million real time data points. Technical details of the fluorimeter survey are presented in an accompanying poster. The analyses of the seawater samples show the concentration of dissolved hydrocarbon, and oil droplets in the surface waters directly beneath the slick. The surface slicks collected from the spill site towards the Alabama, Florida coastline show, through molecular ratios, varying degrees of weathering. In this paper we will present the detailed geochemical findings from both the seawater and surface slicks and compare them with other published data. Particular emphasis will be placed on the interpretation of the weathering and geographical and temporal distribution of these samples.

**RP042 Coagulation/Flocculation Treatment of the Oil Sands Process Affected Water** P. Pourrezacé, Univ of Alberta, Civil and Environmental Engineering.

Coagulation-flocculation (CF) is one of the most commonly used processes to remove suspended solids and colloidal particles with the ability to decrease natural organic matter (NOM) in water and wastewaters. Due to their small size and surface charge, suspended particles cannot be removed by sedimentation or filtration. Thus, they should be destabilized and aggregated into larger particles to be efficiently removed by subsequent treatments. CF can be applied as a pretreatment for the oil sands process affected water (OSPW) generated from the oil sands operations in northern Alberta, Canada. The main constituents of the OSPW include suspended and dissolved solids, metals and organic acids such as naphthenic acids (NAs). NAs are a group of organic compounds with the general formula of  $C_nH_{2n+2}ZO_2$ , in which  $n$  represents the number of carbons and  $Z$  is the number of rings in their structure. In this work, CF was used as a pre-treatment for OSPW. The main objectives were to remove the suspended particles and organic compounds. Aluminum sulfate and ferric sulfate were selected as the main coagulants. In addition, cationic polymer was used as the flocculant. Numerous jar test experiments were performed at various conditions to optimize the process to achieve the highest possible total organic carbon (TOC) removal and turbidity reduction. TOC removal and turbidity reduction were in the range of 7-19% and 90-99% for both aluminum and ferric sulfate. Results for the mixing intensities and times showed that 120 rpm for 30 second and 30 rpm for 10 min were the optimum for rapid and slow mixing periods, respectively. Aluminum sulfate at 250 mg/L was chosen as the optimum concentration to achieve the satisfactory TOC and turbidity removal. Addition of the cationic polymer did not enhance the removal efficiency. The obtained results indicated that coagulation/flocculation using alum was effective treatment for complete turbidity removal which might be beneficial for the advanced oxidation processes. Additionally, satisfactory TOC removal was also achieved which could decrease the load of the organic compounds for the subsequent treatment processes.

**RP043 Aerosol-mediated Transport and Deposition of Brominated Diphenyl Ethers to Antarctica** R.M. Dickhut, Virginia Institute of Marine Science; A. Cincinelli, Università degli Studi di Firenze, Dipartimento di Chimica; M. Cochran, Virginia Institute of Marine Science; H. Kylin, Linköping Univ, Dept of Thematic Studies – Water and Environmental Studies.

Brominated diphenyl ethers (BDEs – 47, 99, 100, and 209) were measured in air, snow and sea ice throughout western Antarctica between 2001 and 2007. BDEs in Antarctic air were predominantly associated with aerosols and were low compared to remote regions of the northern hemisphere, except in Marguerite Bay following the fire at Rothera research station in Sept. 2001, indicating that this event was a local source of BDE209 to the Antarctic environment. Aerosol BDE47/100 reflects a mixture of commercial pentaBDE products; however, BDE99/100 is suggestive of photodegradation of BDE99 during long range atmospheric transport (LRAT) in the austral summer. BDEs in snow were lower than predicted based on snow scavenging of aerosols indicating that atmospheric deposition events may be episodic. BDE47, -99, and -100 significantly declined in Antarctic sea ice between 2001 and 2007; however, BDE209 did not decline in Antarctic sea ice over the same time period. Significant losses of BDE99 and -100 from sea ice were recorded over a 19 day period in spring 2001 demonstrating that seasonal ice processes result in the preferential loss of some BDEs. BDE47/100 and BDE99/100 in sea ice samples reflect commercial pentaBDE products, suggesting that photodegradation of BDE99 is minimal during LRAT in the austral winter.

**RP044 Seasonal Variations of Pharmaceuticals in a River/Lake System in Eastern Finland** A. Meierjohann, Abo Akademi Univ, Laboratory of Organic chemistry; J. Brozinski, Abo Akademi Univ, Laboratory of Organic chemistry; L. Kronberg, Abo Akademi Univ, Laboratory of Organic chemistry.

The river Rakkolanjoki in eastern Finland receives water from the wastewater treatment plant (WWTP) of the city of Lappeenranta (72000 inhabitants). The river is approximately 3 – 10 m wide and consists essentially entirely of treated wastewater from the WWTP. About 15 km downstream from the WWTP, the river enters a small eutrophic lake. At the opposite side of the lake the water enters a small river that reaches the Russian border within a distance of 6 km. The river/lake system was sampled at 9 points and the concentration of 17 different pharmaceuticals including the four antidepressants, were determined by LC – MS/MS analysis following solid phase



extraction. The measured concentrations were normalized against the concentration of the stable wastewater marker compounds carbamazepine and acesulfame. In most cases, the concentrations of the pharmaceuticals in the river were higher than those reported in previous studies and ranged from around 10 ng/l for the antidepressives citalopram sertraline and venlafaxine to close to two µg/l for the antiepileptic drug carbamazepine. For most compounds the transformation rate during the sampling periods in May and June was faster than in February, even though some compounds such as the beta blockers sotalol and bisoprolol did not follow this trend. Normalization of the results against carbamazepine and acesulfame did not provide the expected data on dilution and transformation rates or mechanisms. This may be due to the long residence time in the river/lake system combined with fluctuation in the input of pharmaceuticals.

**RP045 Antimicrobials in Streams/Rivers Subject to Both Agricultural and Urban Influence** A.J. Cessna, National Water Research Institute, Environment Canada, Environmental Health National Program, Agriculture and Agri-Food Canada; C. Yost, Univ of Regina. Frequently, irrigation water is derived from rivers impacted by manure and, when enteric pathogens are present in fecal material, human infections can result. Up to 75% or more of veterinary antimicrobials can be excreted in the feces and/or urine so that manure-treated land also becomes a source of these chemicals to surface water via runoff. Their simultaneous detection in irrigation water may not only help to determine sources of fecal contamination in agricultural watersheds, but may also serve as a possible indicator of the presence of enteric pathogens. The study site was situated in southern Saskatchewan, Canada and included a portion of the Qu'Appelle River watershed of which > 95% consists of agricultural fields and pasture. Within the study site, the Qu'Appelle River runs through the towns of Lumsden and Craven. The Qu'Appelle River is influenced by its tributary Wascana Creek which receives effluent from the Regina City municipal wastewater treatment plant. Downstream of the confluence with Wascana Creek, water is drawn from the River for irrigation of vegetable crops. Sites along Wascana Creek and the Qu'Appelle River were sampled from May to August/September of 2008 and 2009 using automated water samplers programmed to collect weekly composite samples consisting of subsamples collected every 3 h. In 2010, weekly grab samples were collected above and below the treatment plant. After solid-phase extraction, sample extracts were analyzed by LC-MS-MS to determine concentrations of erythromycin (ERY), monensin (MON), lincomycin, tylosin, sulfamethazine and chlortetracycline. Only ERY (30 – 317 ng L<sup>-1</sup>) and MON (4 – 47 ng L<sup>-1</sup>) were consistently detected in the water samples. In Wascana Creek water, concentrations of ERY, which is used in human and veterinary medicine, were significantly higher downstream of the wastewater treatment plant whereas concentrations of MON, which is used only in veterinary medicine, were relatively unchanged. There was a strong correlation ( $R^2 = 0.81$ ) between ERY concentrations just downstream of the treatment plant and those just upstream of the confluence with the Qu'Appelle River suggesting that concentrations in Wascana Creek were dominated by ERY inputs from the treatment plant. In both years, at sampling sites on the Qu'Appelle River where the water is used for irrigation of vegetable crops, concentrations of ERY and MON were lower, most likely because of dilution of Wascana Creek water with Qu'Appelle River water.

**RP046 Chemical Hydrolysis Is a Significant Degradation Pathway for Beta-lactam Antibiotics Compared to Sulfonamide, Amphenicol and Macrolide Antibiotics** S.M. Mitchell, Washington State Univ, Civil and Environmental Engineering; J.L. Ullman, Univ of Florida, Agricultural and Biological Engineering; R.J. Watts, Washington State Univ, Civil and Environmental Engineering. Anthropogenic antibiotic contamination in natural systems presents a human health concern because of the increasing cases of antibiotic-resistance observed over the past few decades. Antibiotics are biologically active at trace levels, and environmental health concerns exist because of the continued biological activity of non-metabolized, excreted compounds released to soil and aquatic ecosystems. About 5-75% of an antibiotic dose is excreted unchanged for the target antibiotics in this study. Therefore, there is a need to elucidate specific antibiotic dissipation mechanisms in natural systems exposed to municipal or agricultural waste effluents. This project focused on hydrolysis reactions, which are fundamental degradation or dissociation reactions caused by water molecules. Two primary factors that affect hydrolysis degradation rates are pH and temperature. In this study, 14 antibiotics (ampicillin, cefalothin, cefoxitin, ceftiofur, chloramphenicol, florfenicol, spiramycin, tylosin, sulfachloropyridazine,

sulfadiazine, sulfadimethoxine, sulfamethoxazole, sulfamethazine and trimethoprim) were separately placed in water at different pH (4-9) and temperature (25°C, 50°C). Degradation was monitored, and first-order rate constants and half-lives were calculated for the compounds that hydrolyzed. This research found that trimethoprim and the selected amphenicol, macrolide and sulfonamide antibiotics did not hydrolyze at neutral pH and 50°C. Notable acid- and/or base-catalyzed hydrolysis was observed for the amphenicol and macrolide antibiotics. However, the four β-lactam antibiotics displayed accelerated hydrolysis at all pH units, with the highest degradation rates at basic pH. Cefalothin and cefoxitin hydrolyzed the quickest with half-lives of less than 10 days at neutral pH and 25°C. Ampicillin and ceftiofur half-lives were about 26 and 53 days at neutral pH and 25°C, respectively. In addition, for the β-lactam antibiotics, second-order rate constants for the acid- and base-catalyzed reactions were computed which can be used to estimate hydrolysis rates at any pH between 4 and 9. Arrhenius coefficients were also calculated which can be used to extrapolate theoretical hydrolysis rates at any temperature. In conclusion, this research suggests that because the β-lactam antibiotics hydrolyzed on the order of days to months at ambient temperatures and neutral pH, they might be less persistent in the environment.

**RP047 Degradation of Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5) in Aquatic Sediments** S. Xu, Dow Corning Corporation, Health and Environmental Sciences; J. Miller, Dow Corning Corporation; R. Gerhards, Evonik Goldschmidt GmbH. As the major ingredients in personal care products, cyclic volatile methylsiloxanes (cVMS) such as D4 and D5 may be released to aquatic environment through waste water treatment discharge and may partition into freshwater sediment. Accurate determination of degradation rates of those compounds in aquatic sediment systems is very challenging due to their high volatility and the difficulty to keep the test material in the aquatic system. In order to overcome the challenge, a new test method was developed for those volatile compounds based on several modifications of the testing guideline (OECD 308 TG by OECD, 2002) to maximize the recovery of the spiked radioactivity of the test material. Using the new methodology, the degradation rates of 14C-D4 and D5 in aquatic sediment systems were determined under both aerobic and anaerobic conditions. The overall average recovery ranged from 88.8% to 105.1% for D4 under various conditions with incubation times ranging from 28 days to 200 days. Generally, D4 undergo hydrolytic degradation in aquatic sediment systems under both aerobic and anaerobic conditions. The degradation rates varied greatly with sediment types, cVMS types and aerobic and anaerobic conditions. Under aerobic conditions, the hydrolysis of D4 was biocatalyzed with half-lives of D4 at 24 oC varied from 47 days in low OC sediment to 242 days for high OC sediment. The sterilization significantly slowed the degradation under this condition. Under anaerobic conditions, the degradation was mostly abiotic with the half-life of D4 in Lake Pepin sediment about one year with no significant difference in measured rates between sterilized and non-sterilized samples. Similar results were found for D5 except that the degradation rates of D5 were slower with an aerobic half-life about 5 years and anaerobic half-life greater than 5 years in Lake Pepin sediment at 24 oC.

**RP048 Organic Carbon Parameterization in Fate and Transport Models of Contaminated Sediment Sites** U. Kipka, P. Israelsson, P. Oates, J. Connolly, D. Chiavelli, C. Forrest, J. Benaman, Anchor QEA, LLC. The fraction organic carbon (foc) in sediment is generally expected to vary by grain size, with finer particles (e.g., clays, silts, fine sands) typically exhibiting greater organic content. The need to represent this variation in foc among particle sizes within contaminant fate and transport models has grown over the past decade as sediment transport calculations involving multiple size classes have become more common. In constructing a coupled hydrodynamic, sediment transport, and polychlorinated biphenyl (PCB) fate and transport model of the Upper Hudson River (UHR) PCB Superfund Site, we have noted the influence of foc parameterization in several contexts, including the prediction of downstream PCB load following flow-driven sediment resuspension during storm events. In these cases, accurately defining foc values for each size class is important primarily because it controls partitioning behavior within the sediment bed, and thus the concentration distribution among the different size classes of eroding particles. Sediment cores from the extensive UHR 2003 Sediment Sampling and Analysis Program database were analyzed using several approaches to estimate a representative relationship between foc and particle size. Class-specific foc values

were determined for each of the four modeled sediment size classes for incorporation into the UHR PCB fate and transport model. In this work, foc analysis and parameterization approaches will be presented along with illustrations of model response. Although the UHR application has the benefit of a large dataset to constrain site specific foc values, the considerations reviewed in this talk have general relevance to the simulation of chemical fate and transport at contaminated sediment sites.

**RP049 Investigating the Environmental Fate, Dose-Response and Toxicokinetics of Triclosan in *Daphnia* spp.** K. Albanese, The Ohio State Univ Environmental Science Graduate Program; R. Lanno, The Ohio State Univ Dept of Evolution, Ecology and Organismal Biology; Y. Chin, The Ohio State Univ School of Earth Sciences; C. Hadad, The Ohio State Univ Dept of Chemistry. Pharmaceuticals and personal care products (PPCPs) have recently become an increasing environmental concern as their usage has escalated dramatically and more is discovered about their potential toxic and estrogenic effects. One common PPCP, triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether), is an antimicrobial compound that has recently been included in commercial toothpaste, hand soap, and deodorant formulations and has been classified as an "emerging organic pollutant". With a relatively high log  $K_{ow}$  (4.8), triclosan may bioaccumulate in organisms. In vitro assays have also shown triclosan to impair mitochondrial function, activate pregnane X receptors, and inhibit estrogen and thyroid hormone sulfotransferases in humans and has demonstrated toxicity to algae. A more detailed understanding of the environmental fate of triclosan is needed since, in addition to concerns associated with toxicity of the parent compound, triclosan has been shown to transform to various forms of dioxin in aquatic systems. Little is known about how this process occurs or the rate and the extent to which it occurs under varying aquatic conditions, i.e., DOC concentration. This study will examine possible reaction intermediates and transition states of triclosan in water by both laboratory experiments and computational chemistry, determining the bioavailability of triclosan to *Daphnia* spp. via bioaccumulation tests and reproduction bioassays, and determining the toxicokinetics of triclosan. The effects of DOC concentration on these processes will also be examined.

**RP050 Contaminants in Fish and Sediment of the Charles River Near Boston, USA, 2005** K. Echols, T. May, US Geological Survey, Columbia Environmental Research Center; P. Peterman, US Geological Survey; J. Meadows, K. Feltz, W. Brumbaugh, C. Orazio, US Geological Survey, Columbia Environmental Research Center. The Charles River watershed drains 300 square miles including 24 cities and towns. Common carp, largemouth bass, white suckers, and sediments from the Watertown Dam to New Charles River Dam reach in the Boston area were collected as part of USGS study evaluating effects of contaminants on fish populations. Individual whole fish and sediment samples were extracted, purified and fractionated for polycyclic aromatic hydrocarbon (PAH), polychlorinated biphenyl (PCB), organochlorine pesticide, and polybrominated diphenylether (PBDE) residue analyses by dual column GC-ECD or GC/MS. The fish and sediments were also analyzed for Ni, Cu, Zn, Cd, Pb, Hg, As, Se, and ICP-MS elements scan. Sediment analyses included acid volatile sulfide (AVS) and simultaneously extractable metals. Complex mixtures of organic contaminants were found in the fish at the following concentration ranges (ng/g wet weight): total-PCBs (960-3600), total-PBDE (50-400), total-toxaphene (< 19-100); dieldrin (6-210) and p,p'-DDE (100-500). PBDE congeners 47, 100, and 28 were predominant in the fish, with PBDE-47 comprising 40-70% of the total-PBDE. Total-PCBs in the three sediment samples were 110, 3400, and 5000 ng/g dry weight; sediment levels of chlordane and DDT related components were higher than the other pesticides. Total-PBDE concentrations in all sediment samples were < 6 ng/g. Sediment had high levels of PAHs, ranging up to 20,000 ng/g dry weight for some individual PAHs. PAH concentration ratios indicated both pyrogenic and petrogenic sources. Tetra-octa congeners of polychlorinated terphenyls (PCT) were also present in the sediments. Sediment concentrations Ni, Cu, Zn, Cd, Pb, Se, and As were unremarkable. Fish filets had mercury levels ranging from 2.48 to 4.43 µg/g dry weight, exceeding the USEPA consumption advisory for edible fish portions of 1.5 µg/g dry weight (0.3 µg/g wet weight). In summary, the mixture of contaminants in Charles River fish and sediments are indicative of multiple sources: storm water runoff, municipal waste water discharge, urban and agricultural pesticides, and industrial effluents.

**RP051 High Resolution Observations of Near-surface Properties in Boston Harbor: Implications for Transport from Land to Ocean** B. Gardner, R. Chen, UMASS Boston, EOS. Multiple observations in Boston Harbor and its tributaries have demonstrated the importance of carefully examining the very near surface layer (top 1 m) within the Harbor, and by extension, other comparable waters. In the early 1990's we conducted "tow-yo" surveys of Boston Inner Harbor using a Seabird 9/11 CTD system. We found a very thin (< 1m) low salinity layer which would be missed by standard sampling techniques. In 2000, Gordon Wallace asked one of us (Bernie Gardner) to survey the Weymouth Fore River to examine nutrient concentrations along a horizontal salinity gradient. The plan was to slowly tow the Seabird CTD at the surface and take samples approximately every 2 PSU salinity value. That turned out to be impossible because the surface salinity was essentially constant (and low) well into the harbor, and then at the edge of the "plume" suddenly increased over a scale of a few meters. More recently, we have found that under common river flows there is essentially a salt wedge in the upper stretch of the Neponset River with the bulk of the upper mixed layer found between 0.5 and 1.0 meters. Colored dissolved organic matter (CDOM) can provide a good tracer of the transport of land-derived dissolved compounds through the Harbor into the coastal ocean. Generally, the largest source of CDOM comes from land drainage associated with decaying plant material, though we have found significant contributions from salt marshes. We will present results of our studies using towed vehicles and moored instruments to provide high temporal and spatial measurements of the near surface environment and demonstrate the importance of a thin surface layer to the flux of dissolved material from the land to the coastal ocean.

**RP052 Seasonal Land Use and Land Cover Effects on Nutrient Levels of the Neponset River Watershed** N. Henderson, A.D. Christian, Univ Of Massachusetts Boston, Biology Dept; R. Hannigan, Univ Of Massachusetts Boston, Environmental, Earth, and Ocean Sciences. The Neponset River Watershed is a 337 km<sup>2</sup> urban watershed located in Boston, MA and flows into the recently restored Boston Harbor. While Boston Harbor has undergone extreme restoration, the streams flowing into the harbor largely have been ignored. The goal of this research was to compare physical and chemical parameters of at 5 different land cover/use treatments (forested, golf course, industrial, residential, and wetland) in the watershed. Water samples were collected monthly from September 2009 to September 2010 from 33 sites. Each sample was measured for temperature, DO, conductivity, pH, TSS, AFDM, nitrate, ammonium, orthophosphate, TDN, TDP, and particulate C, N, and P. Using MANOVA analysis, we found that conductivity, DO, pH, temperature, and TSS were statistically different among land use treatments. We conclude that both seasons and land uses appear to influence water quality in the Neponset River Watershed and that implementation of best management practices could increase water quality in the watershed.

**RP053 Towards Rational Design of Safer Chemicals: Property Guidelines for Reduced Acute Aquatic Toxicity** A. Voutchkova, Yale Univ, Center for Green Chemistry and Green Engineering, Yale Univ; J. Zimmerman, Yale Univ, Center for Green Chemistry and Green Engineering; P. Anastas, Yale, Center for Green Chemistry and Green Engineering; B.W. Brooks, Baylor Univ, Environmental Science and Biomedical Studies, Baylor Univ, Dept of Environmental Science. One of the most elusive yet significant goals of green chemistry is for chemists to routinely design commercially useful chemicals with reduced toxicological hazard. The main objective of this study was to derive property guidelines for the design of chemicals with reduced acute aquatic toxicity. The properties explored included chemical solubilities, size, shape and molecular orbital energies. Physicochemical properties were predicted using Schrodinger's QikProp, while frontier orbital energies (HOMO, LUMO and HOMO-LUMO) were determined based on AM1 and DFT calculations using Gaussian03. Experimental toxicity data included acute toxicity thresholds (LC<sub>50</sub>) for the fathead minnow (*Pimephales promelas*; 570 compounds), the Japanese medaka (*Oryzias latipes*; 285 compounds), a cladoceran (*Daphnia magna*; 363 compounds) and green algae (*Pseudokirchneriella subcapitata*, 300 compounds). Mechanistically-driven qualitative and quantitative analyses between the in-silico predicted molecular properties and in-vivo toxicity data were explored in order to propose property limits associated with higher probabilities acutely safe chemicals. The analysis indicates that 70-80% of the compounds that have low or no acute aquatic toxicity concern to the four species have a defined range of values for octanol-water partition

coefficient ( $\log P_{o/w}$ ) and  $\Delta E$  (LUMO-HOMO energy). Compounds with  $\log P_{o/w}$  values less than 2 and  $\Delta E$  greater than 9 eV are significantly more likely to have low acute aquatic toxicity compared to compounds that do not meet these criteria. These results are mechanistically rationalized. Our work proposes design guidelines that can be used to significantly increase the probability that a chemical will have low acute toxicity to the four species studied, and potentially other aquatic species.

**RP054 A Novel, Safer Treatment of Liver Cancer Based on Carbon Nanotubes Functionalized with Bovin Serum Albumin** L. Mocan, T. Mocan, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Nanomedicine Dept; D. Bartos, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania; F. Zaharie, R. Stiufuc, A. Stir, C. Iancu, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Nanomedicine Dept. We previously presented a method of Carbon nanotube-enhanced laser thermal ablation of HepG2 cells (Human hepatocellular liver carcinomacell line), based on a simple multi-walled carbon nanotube (MWCNT) carrier system, such as human serum albumin (HSA), and demonstrate its selective therapeutic efficacy. Transmission electron, contrast phase and confocal microscopy combined immunochemical staining were used to demonstrate the selective internalization of HSA-MWCNTs via Gp60 receptors and the caveolin-mediated endocytosis inside HepG2 cells. We examined the ability of laser activated carbon nanotubes to induce Hsp70 expression using confocal microscopy. Hep G2 cells heat-shocked (laser activated HSA-MWCNTs) to 42°C demonstrated an up-regulation of Hsp70 compared with control cells (HSA-MWCNTs treated cells without laser), which showed no detectable constitutive expression of Hsp70. We observed a time-dependent induction in Hsp70 expression in Hep G2 treated with HSA-MWCNTs and LASER irradiated. The post-irradiation apoptotic rate of HepG2 cells treated with HSA-MWCNTs ranged from 88.24% (for 50 mg/L) at 60 seconds, while at 30 minute the rate increased to 92.34% (50 mg/L). These unique results may represent a major step in liver cancer treatment using nanolocalized thermal ablation by laser heating.

**RP055 Biodegradable Solvents for the Pharmaceutical Industry: A Study of Toxicity, Ecotoxicity and Stereochemistry of (a) Chiral Ionic Liquids** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences; D. Coleman, Dublin City Univ, School of Chemical Sciences and National Institute for Cellular Biotechnology. The search for more environmentally friendly alternatives to Volatile Organic Compounds (VOCs) has become an important issue in recent years. Ionic Liquids (ILs) have been studied as possible replacements for these solvent types. ILs are highly polar solvents with negligible vapour pressure and low flammability that offer a potentially "green" alternative to VOCs. However in order to confidently label this class of solvents as "green", their behaviour in the environment must be thoroughly examined. As a result various toxicity, ecotoxicity and biodegradation studies have been carried out on ionic liquids. A number of (a)chiral ILs have been designed and synthesized where functional groups to improve biodegradation are incorporated into the cation side chain. Toxicity and biodegradation studies have been carried out on the ILs, to investigate their potential as green solvents. In the toxicity screening, the ILs were tested against several strains of bacteria using a Minimum Inhibitory Concentration (MIC) assay. An activated sludge assay was also employed to investigate the biodegradability of the ionic liquids. HPLC-MS analysis was used to determine the primary biodegradation and subsequently determine the possible metabolites formed during biodegradation. The results obtained from these tests have proved promising and are in accordance with literature.

**RP056 Formation and Structure Elucidation of Stable Transformation Products of Pharmaceuticals in the Water Cycle** M. Lamoree, Institute for Environmental Studies, VU Univ, Chemistry & Biology, Institute for Environmental Studies, Chemistry & Biology; E. Baginska, T. Haddad, C. Leder, Leuphana Universität; P. Roche, V. Boireau, A. Hebert, Veolia Environnement Recherche et Innovation SNC; B. Roig, S. Mompelat, Ecole des Hautes Etudes en Santé Publique; K. Kuemmerer, Leuphana Universität. Pharmaceutically active substances can undergo transformations starting from human metabolism to degradation in advanced effluent treatment, in environmental processes and finally during drinking water treatment. Often degradation in sewage and water treatment and the environment is incomplete, resulting in the formation of stable transformation products. In only a few cases, full mineralization of the parent compounds is achieved. This

is even more important as advanced oxidation techniques employing e.g., ozone, hydrogen peroxide, light or electro-coagulation are subject of discussion for the removal of pharmaceuticals in effluent treatment and drinking water treatment. Treatments using these techniques may even lead to the formation of transformation products that are more toxic than the parent compound. In the past years, comprehensive information on the degree of contamination of our environment with pharmaceuticals has been collected, but there are still some important knowledge gaps regarding the ecological and human health impact of two groups of pharmaceuticals, which will be addressed in the Pharnas project (EU grant agreement no. 265346). On the one hand, for anti-cancer drugs only little information is available on environmental concentrations, while for the antibiotics, concern is raised regarding their occurrence and the potential role of these compounds in spreading resistance against these valuable pharmaceuticals. For a selection of pharmaceuticals of both classes, the formation of stable transformation products in various stages of the water cycle (both drinking and sewage) will be investigated in laboratory and pilot scale studies. Treatment processes include mainly chlorination, ozonation and UV-disinfection for drinking water treatment and advanced oxidation, photolysis/photocatalysis/photofenton for sewage treatment. For structure elucidation of the transformation products formed, different LC-MS/MS approaches as well as high resolution MS techniques will be implemented. Characterization of the stable transformation products in terms of (eco)toxicological behavior will be done using a battery of in vitro and in silico bioassays. The knowledge on specific transformation product formation pathways, the molecular identity and (eco)toxicological behavior is expected to ultimately lead to recommendations for the targeted design of pharmaceuticals with improved degradation and elimination properties, whilst maintaining their therapeutic value.

**RP057 Highly Recyclable, Low Antimicrobial and Antifungal Toxicity Ionic Liquids: A New Strategy for Brønsted Acid Catalysed Reaction** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences; R. Gore, Dublin City Univ, School of Chemical Sciences and National Institute for Cellular Biotechnology. The research project combines two fields of green chemistry, ionic liquids and catalysis. Our study is directed towards ionic liquids which can also catalyse reactions. This overlap between organocatalysis and ionic liquid research enables us to design low toxicity and potentially biodegradable catalysts based on the extensive biological screening data of ionic liquids. Assessment of the performance of a new catalyst, in tandem with the (eco) toxicity screening, allows the chemist to develop green synthetic methods. We have designed a library of aprotic ionic liquids which can act as Brønsted acid catalysts with low antibacterial and antifungal activity. Preliminary studies of the toxicity of all the ionic liquids were performed to establish the influence of the ester or amide group in the cation. Antifungal and antibacterial toxicity studies demonstrated that the ionic liquids did not inhibit the growth of any organism screened at concentrations of 2.0 mM. Acetalisation and thioacetalisation reactions of a variety of aldehydes have been studied at room temperature and low catalyst loadings. The study demonstrated the activity of these aprotic molecules as active Brønsted acids in the presence of protic media, as well as the importance of the choice of counter-ion in catalytic activity. The most active catalyst has been recycled 15 times without any loss of catalytic activity.

**RP058 Selective Hydrogenation of Trans-cinnamaldehyde and Hydrogenolysis-free Hydrogenation of Benzyl Cinnamate in Imidazolium ILs** N. Gathergood, Dublin City Univ, Dublin City Univ, School of Chemical Sciences, Dublin City Univ, Chemical Sciences; M. Gurbisz, Dublin City Univ, School of Chemical Sciences and National Institute for Cellular Biotechnology. Applications of ionic liquids as solvents in clean hydrogenation reactions under very mild conditions were studied. Reductions of olefins in the presence of other functional groups with metal catalysts were performed, at various catalyst loadings and study of catalyst recycling. Remarkable results were recorded for selective hydrogenation of trans-cinnamaldehyde to the saturated aldehyde and reduction of benzyl cinnamate without hydrogenolysis of the benzyl ester both at 100% conversion. Asymmetric reduction of methyl  $\alpha$ -acetamidocinnamate in the presence of chiral catalysts was also studied.

**RP058.5 Designing Molecular Guidelines for Reducing the Acute Toxicity of Pesticides on Birds Using Support Vector Regression** B. Husowitz, Center for Green Chemistry and Green Engineering at Yale. There



is a definite need to develop QSAR methods to help with the rational design of chemicals that have reduced toxicological hazard. In this regard, support vector regression SVR methods were used to derive molecular guidelines that will reduce the acute toxicity of pesticides on birds. The chemical descriptors used in the QSAR study were predicted using Schrodinger's QikProp and Epik programs along with various Density Functional Theory (DFT) based descriptors. SVR methods enabled us to identify ranges and bounds for various chemical properties. These bounds and ranges define the necessary chemical properties that a pesticide should possess to be considered nontoxic for a wide range of bird species. These species include, but are not limited to, Mallard Duck, Canada p Goose, Japanese Quail, Domestic Chicken, Ring-Necked Pheasant.

**RP059 Ecotoxicological Evaluation for Metal Contaminated Abandoned Mine Soil in Korea** J. Son, Y. Kim, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; Y. Lee, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, Environmental Science and Ecological Engineering; M. Kim, Korea Univ; S. Hyun, Korea Univ, Environmental Science & Ecological Engineering; K. Cho, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, # 407, Division of Environmental. Mining activities may be one of significant sources of metal contamination of soil as well as sediment. From the regulatory perspectives, ecotoxicological tests coupled with analytical techniques have been gaining popularity as supplementary tools to assess ecotoxicological risk caused by chemicals to human and environmental health. In this study, soil toxicity tests using microbe (microbial enzyme activities) and invertebrate (collembola reproduction) as well as sediment toxicity tests using bacteria (*Vibrio fischeri*) and crustacean (daphnid/ostracod survival) were carried out to assess ecotoxicity of metal contaminated mine soil. The reproduction toxicity test was conducted with collembola, *Paronychiurus kimi* using soil as test medium. The Microtox solid-phase test was performed with *V. fischeri*, and sediment toxicity tests with *Daphnia magna* and *Heterocypris incongruens* were conducted in accordance to the ASTM guideline. From the results of this study, all the toxic responses were closely related to the metal concentration in the mine soil, regardless of test species. These results clearly demonstrate that the use of ecotoxicity test coupled with chemical analysis is promising way to assess ecological risk and should be incorporated in the context of ecological risk assessment process.

**RP060 Mass Balance Assessment of Dioxins in Kaolin Ball Clay Used in Ceramic Industry** Y. Horii, N. Ohtsuka, K. Minomo, K. Nojiri, Center for Environmental Science in Saitama. Elevated concentrations and unique congener profiles of dioxins in American ball clay were attributed to arise from the natural formation processes. Several studies have been conducted to elucidate the sources and mechanisms of formation of high concentrations of dioxins, particularly polychlorinated dibenzo-*p*-dioxins (PCDDs; >450 ng/g dry wt and 1500 pg WHO-TEQ/g dry wt) in ball clays. An estimated 1.1 million metric tons of American ball clay was mined in 2007, for use in the ceramic industry. Our recent study has reported that inventory of dioxins from kaolin clay production worldwide was estimated to be 650 kg/yr on a mass basis and 2400 g-TEQ/yr on a toxic equivalents. In the production of ceramics, dioxins present in kaolin can be destroyed or volatilized at high temperature (typically exceed 1000 °C in kilns). In this study, we investigated the mass balance assessment of dioxins in ceramic industry based on laboratory experiment. Two gram each of American ball clay was baked at the constant temperatures from 200 °C to 1000 °C for 1h with continuous nitrogen flow (400 mL/min) in a quartz tube installed in a muffle furnace. Flue gas from baked ball clay was collected using a gas sampling unit (water and diethylene glycol absorption bottles, and XAD-2 adsorbent); this connected to the outlet of quartz tube. Concentrations of dioxins were determined both in the flue gas and residue of ball clay using a gas chromatography-high resolution mass spectrometry, and rates of destroyed and volatilized dioxins during kiln were estimated. In preliminary results, on a mass basis, 20% of dioxins reduced in the residue of ball clay whereas negligible level of dioxins was observed in the flue gas after kiln at 200 °C. After kiln at 600 °C, non-detectable dioxin was found in the residue of ball clay, and 5% of dioxins was observed in the flue gas, indicating that most of dioxins in ball clay was destroyed at high temperature. We are currently investigating the volatilization rate of dioxins from baked ball clay at the temperature gradually increase from 100 °C to 1000 °C, which is

more reliable to the actual conditions of firing clay in ceramic industry, and estimating the emission of dioxins from ceramic industry.

**RP061 Assessment of Natural Background Levels and Composition of Dioxins in Mississippi River Sediment** A. Pawlisz, Conestoga-Rovers&Associates. There is mounting evidence documenting the natural formation and deposition of polychlorinated dibenzo-*p*-dioxins (PCDDs) dating back to middle Eocene (40 to 45 million years ago) in the Mississippi River (River) watershed. The initial discovery of elevated levels of non-anthropogenic dioxins in soils along the River came as part of a nationwide survey of food conducted by the United States Food and Drug Administration (FDA). The agency detected high levels of dioxins in chickens fed soybean meal containing fine-grained hydrated aluminum silicate (ball clay). The clay was used as an anticaking additive, which was determined to originate from a quarry in Crenshaw, MS. A second dioxin-affected clay was discovered by the US Dept of Agriculture (USDA) and the US Environmental Protection Agency (USEPA) in Sledge, MS. Both mines were found in an area associated with a geologic formation called Mississippi Embayment (ME), which spans over 45,000 square miles along the River. Subsequent investigations by the USEPA demonstrated conclusively that the ball clay from these mines was the source of dioxins found in animal stock and feed. This determination was based on the strong correlation between the clay and feed/animal tissue concentrations, isomer ratios, and dioxin patterns. The presentation discusses the results of key dioxin studies and provides a synopsis of recent evidence documenting the natural formation and deposition of PCDDs in the vicinity of the River. The significance of the findings is discussed in context of fingerprinting methods and characteristics of natural vs. anthropogenic dioxins as applicable to assessing background levels of dioxins in the River sediments.

**RP062 Dioxin-like Toxicity in Sediments and Floodplain Soils from the Saginaw River Watershed: Polychlorinated-dibenzo-*p*-dioxins, Dibenzofurans, Biphenyls** S. Yun, Wadsworth Center, Student, New York State Dept of Health; K. Kannan, Wadsworth Center, New York State Dept of Health; J.M. McCabe, A. Ostaszewski, A.B. Taylor, D.M. Taylor, Waste and Hazardous Materials Division, Michigan Dept of Environmental Quality. Concentrations of PCBs including non-ortho coplanar congeners, PCDDs, and PCDFs were measured in more than 120 sediment and floodplain soil samples collected from the Shiawassee River (a tributary of the Saginaw River), the Saginaw River, and Saginaw Bay, to determine the sources and magnitude of contamination, and to elucidate the contributions from individual contaminant groups to the overall 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs). Sediment and soil extracts were also analyzed for total dioxin-like activity by means of the H4IIE-luc cell bioassay. Elevated concentrations of PCBs (>1000 ng/g, dry weight) were found in the Shiawassee River near Chase Lake, from Middleground Island in the Saginaw River, and from subsurface sediments in Saginaw Bay. Concentrations of PCDD/Fs from the Saginaw River and Saginaw Bay were 2-3 orders of magnitude higher than concentrations in the samples from the Shiawassee River. The highest PCDD/F concentration (55200 pg/g, dry weight in a subsurface layer) was found in sediment collected at the mouth of the Saginaw River. Concentrations of PCDFs were greater than the concentrations of PCDDs in sediment from the Saginaw River. 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF were the major PCDF congeners found in sediments from the Saginaw River. The elevated concentrations of PCDFs, and the predominance of the less highly chlorinated PCDF congeners, in sediments from the Saginaw River were similar to previously determined characteristics of the PCDF contamination of the Tittabawassee River. These results suggest the existence of a major source of PCDFs within the watershed. A few localized areas of high PCDD/F and PCB concentrations, with unique congener compositions, in the Saginaw River indicated the presence of other minor sources, such as wastewater treatment plants. PCDFs were the major contributors to TEQs from the Saginaw River and Saginaw Bay. Approximately 30% of the samples analyzed in this study had values exceeding the screening level of 50 pg TEQ/g, dry wt, suggested for soils by the Agency for Toxic Substances and Disease Registry.

**RP063 Sources and Distribution of Polychlorinated-dibenzo-*p*-dioxins and Dibenzofurans in Soil and Sediment from the Yellow Sea Region of China and Korea** J.E. Naile, Univ of Saskatchewan, Dept of Veterinary Biomedical Sciences and Toxicology Centre; J. Khim, Korea Univ, Division of Environmental Science and Ecological Engineering; T. Wang, Chinese

Academy of Science, Research Center for Eco-environmental Sciences; Y. Wan, Peking Univ; S. Hong, Korea Univ, Division of Environmental Science and Ecological Engineering; ; P. Jones, Univ of Saskatchewan, School of Environment and Sustainability; ; Y. Lu, Chinese Academy of Science, Research Center for Eco-environmental Sciences; J. Giesy, Univ of Saskatchewan, Dept of Veterinary Biomedical Sciences and Toxicology. Polychlorinated-dibenzo-*p*-dioxins and -dibenzofurans (PCDD/Fs) were measured in soils and sediments from the Yellow Sea region. Korean soils and sediments mostly contained detectable PCDD/Fs and showed a widespread distribution among locations. Soil and sedimentary PCDD/Fs from China were comparable to or less than those in Korea. The patterns of relative concentrations of individual congeners in soils were different between the two countries, but similar in sediments. Sources of PCDD/Fs in China and Korea were found to be independent of each other and their distributions reflected matrix-dependent accumulation. Spatial distribution indicated some point sources in Korea while Chinese sources were more widespread and diffuse. PCDD/Fs measured in the coastal areas of the Yellow Sea were comparable to or less than those previously reported in for eastern Asia. When compared to Canadian guidelines, which are one of the more stringent guidelines, 51% of Korean soils ( $n = 49$ ) and 79% of Chinese soils ( $n = 48$ ) did not exceed corresponding guidelines, but all Japanese soils ( $n = 20$ ) exceeded the guidelines.

**RP064 Analysis of Perfluorochemicals and Pharmaceuticals in Fish Using ACQUITY Liquid Chromatography-Xevo G2 QToF Mass Spectrometry** T.M. Holsen, B. Crimmins, X. Xia, Clarkson Univ. The Great Lakes Fish Monitoring Program (GLFMP), administered by the USEPA Great Lakes National Program Office (GLNPO), aims to monitor temporal trends of emerging contaminants in the Great Lakes using top predator fish as biomonitors. Recent work has focused on quantifying the concentrations of perfluoroalkyl compounds (PFCs) and pharmaceuticals in lake trout due to their environmental persistence, toxicity and potential chronic effects on the aquatic environment. In this study, an ultra-high pressure liquid chromatography-mass spectrometry (UPLC-MS) method was developed. PFCs and pharmaceuticals in fish tissue were quantified using a Waters Xevo G2 QToF UPLC-MS. Reversed-phase separation of target compounds was achieved using an ACQUITY UPLC<sup>®</sup>HSS T3 column for both classes of compounds using aqueous 0.1% (V/V) formic acid and methanol mobile phases. The mass spec was configured to continuously monitor precursor and product ions to selectively quantify compounds of interest and identify potential interferences respectively, utilizing Waters MSe technology allowing providing precursor/product identification for the entire chromatogram. Sample preparation procedures and analysis methods will be discussed with results of different target compound classes.

**RP065 Mass Spectrometric Identification of Naturally Produced Brominated Compounds in Fish and Fish Oil** A. Covaci, Univ of Antwerp, Toxicological Centre; A.C. DIRTU, Toxicological Centre, Univ of Antwerp, Dept of Pharmaceutical Sciences. While fish products of marine origin are essential for the human diet, their consumption may result in increased exposure to anthropogenic organohalogen contaminants (OHCs), e.g., polybrominated diphenyl ethers (PBDEs). Recently, the presence of naturally-produced organobrominated compounds (NOCs), such as methoxylated PBDEs (MeO-PBDEs) has been reported in the marine environment. Here we present the identification of different classes of NOCs in fish oils dietary supplements and in fish of commercial value (e.g., bluefin tuna). Tetrabrominated MeO-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) were identified based on their retention times and confirmed by EI-MS spectra and comparison with authentic standards. The molecular ion cluster showed an abundant ion at  $m/z$  516, followed by an ion cluster centered at  $m/z$  420, corresponding to  $[M-Br-CH_3]^+$ , specific for an *ortho* substitution of the MeO group. These two compounds were detected in 90 % of FODS and in 100% of fish samples and their concentrations were, in most cases, higher than those of PBDEs. Levels of other tri- to pentabrominated MeO-PBDEs were minor. In many samples, the retention times of two peaks eluting after the tetrabrominated MeO-PBDEs did not match the retention times of available MeO-PBDE standards. For the 1<sup>st</sup> peak, the molecular ion at  $m/z$  468  $[M]^+$  in the ECNI-MS spectra had a low abundance, while the weak fragment at  $m/z$  387  $[M-Br]^+$  contained 2 Br atoms. In the EI-MS spectra, the isotope pattern for the fragment at  $m/z$  464  $[M]^+$  corresponded to three Br atoms. In contrast, the 2<sup>nd</sup> peak showed an abundant molecular ion in ECNI-MS at  $m/z$  542  $[M]^+$ , while fragment ions were found at  $m/z$

466  $[M-Br]^+$ . Likewise, its molecular ion ( $m/z$  546  $[M]^+$ ) containing 4 Br atoms was detected in EI-MS mode, while other important fragment ions were detected at  $m/z$  385  $[M-Br]^+$  (3 Br atoms) and  $m/z$  264  $[M-HBr_2]^+$  (2 Br atoms). Both compounds presented the base ion at  $m/z$  121  $[C_9H_{13}]^+$  originating from a non-brominated fragment. By comparing retention times and mass spectra (ECNI and EI) with reference standards (courtesy of W. Vetter, Germany), the peaks were identified as tri- and tetrabrominated hexahydroxanthenes (PBHDs). PBHDs had a high detection frequency in fish oil (>50%) and fish samples (>95%). Both classes of NOCs have been measured in concentrations higher than OHCs usually targeted in monitoring schemes, but more has to be learned about their occurrence and their potential toxicological effects.

**RP066 Screening for High Molecular Weight Phthalates in Sediments Using Atmospheric Pressure Chemical Ionization GC/MS** S. Lai, K. Rosnack, Waters Corporation; D. Stevens, Waters Corp., GC/MS Marketing Manager; P. Hancock, Waters Corp.; F. David, P. Sandra, Research Institute for Chromatography. Measuring phthalates in environmental samples is an important application due to their potential endocrine-disrupting properties. Most classical methods for this application are based on gas chromatography mass spectrometry (GC/MS). Attention has recently turned to the analysis of di-isononylphthalate (DiNP) and di-isodecylphthalate (DiDP) but their analysis by traditional GC/MS results in an unresolved cluster of peaks that all fragment to the phthalic anhydride ion at  $m/z = 149$ . The difficulty in chromatographically resolving the separate isomeric forms along with the poor selectivity of the abundant fragment at 149Da makes detection and quantitation of individual forms challenging. Consequently, quantification of DiNP and DiDP is impossible at the same sensitivity levels possible for the single isomer phthalates when using conventional GC/MS with electron ionization (EI). Atmospheric pressure ionization has primarily been used to interface MS with LC but it can now also be applied to GC/MS. Atmospheric pressure gas chromatography (APGC) is a novel atmospheric pressure ion source for MS instruments that allows well established chemical ionization (CI) types including protonation, charge exchange and electron capture to be applied to gas phase analytes eluting from a GC column. Due to the source region being at atmospheric pressure during operation and its capability of handling very high gas flow it is possible to employ a wider range of CI reagents including some, such as water, not practical for routine use with vacuum source CI instruments. Furthermore, GC separations can be optimized as the carrier gas flow and column dimensions are not limited by the pumping capacity as is the case for ionization sources that run under a vacuum. In this work, APGC coupled to a quadrupole time-of-flight mass spectrometer was used to analyze high-molecular-weight phthalates. DiNP and DiDP were successfully ionized in protonation mode yielding spectra that contained the molecular ion as the base peak. The method was also applied to measure DiNP and DiDP in a sediment extract at relevant levels for environment samples.

**RP067 Temporal Trends in Human Exposure to Fluorosurfactants and Their Metabolites** L.W. Yeung, S. Robinson, Univ of Toronto, Dept of Chemistry; S.A. Mabury, Univ of Toronto. Fluorinated chemicals have been used in various industrial and daily applications for over 5 decades. Their unique oil-repelling and high surface activity make them excellent surface-protectors and surfactants. Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are two well-known fluorinated chemicals which, due to their persistency and bioaccumulation potential, have been banned in several countries. Another group of fluorinated chemicals having a phosphate head group, polyfluoroalkyl phosphoric acid diester surfactants (diPAPs), has received growing attention. Unlike PFOS and PFOA, diPAPs are direct consumer products, used in food contact paper, while PFOS and PFOA were mainly used in industry. Through consumption of diPAP contaminated food, humans may have direct exposure to these chemicals. Recent studies found diPAPs in North American human blood serum at low part-per-billion (ppb) levels. Such low concentrations make quantification difficult for archived samples of limited size. In the present study, human blood serum samples from the German Environmental Specimen Bank (1981-2009) were analyzed for this new class of fluorinated chemicals using an Agilent 1100 HPLC and AB Sciex API 4000 MS-MS in ESI(negative mode). Preliminary results indicated diPAPs at low ppb levels, and many of them were not quantifiable (lowest point of the calibration curve: 0.5 ng/mL). A new Acquity UPLC and Waters Xevo-TQ S MS-MS system has been used for further analysis. Preliminary results suggest the limit of

quantification is 0.05ng/mL with an injection of 6  $\mu$ L of sample on-column. Results for 300 samples from the German Environmental Specimen Bank will be presented, and challenges towards diPAP analysis will be discussed.

**RP068 Untargeted Screening of San Francisco Bay Harbor Seals for New Organohalogen Pollutants** J.R. Kucklick, National Institute of Standards and Technology (NIST), Analytical Chemistry Division, National Institute of Standards & Technology, Hollings Marine Laboratory; S. Klosterhaus, San Francisco Estuary Institute; N. Dodder, Southern California Coastal Water Research Project; E. Hoh, San Diego State Univ, Graduate School of Public Health; J. Murray, National Institute of Standards and Technology, Analytical Chemistry Division; D.J. Greig, The Marine Mammal Center; K. Maruya, Southern California Coastal Water Research Project. Contaminant measurements are generally made based on target compound lists and calibration standard availability. Many compounds produced by society are not routinely monitored even though several of these compounds have physical properties similar to well known bioaccumulative pollutants. The goal of this study was to use a relatively new analytical tool, two dimensional gas chromatography with time of flight mass spectrometry (GCxGC TOF/MS), to screen harbor seal (*Phoca vitulina*) samples from San Francisco Bay, California for new contaminants. The GCxGC technique allows for separation of several thousand individual compounds in a single run while the TOF/MS portion allows for full electron impact ionization spectra to be acquired. Samples (blubber, liver and blood) were collected from six dead stranded or euthanized harbor seals from locations around San Francisco Bay. In addition, liver and blubber samples were obtained from an archived harbor seal collected from Alaska to serve as a "non-urban" control. Blubber samples were extracted, processed through size exclusion chromatography, and then fractionated by silica gel/alumina chromatography. Serum samples were extracted to yield a neutral and a phenolic fraction. Samples were analyzed by GCxGC TOF/MS. To date, blubber samples have been analyzed. Legacy pollutants tended to dominate with several unusual compounds tentatively identified as DDT-related, current use pesticides, chlorinated polycyclic aromatic hydrocarbons and other halogenated compounds. Results from this work will help to inform water managers in the San Francisco Bay region about the scope of contamination in the bay as reflected in higher organisms.

**RP069 Review of Tissue Effect Concentrations for Mercury in Benthic Invertebrates** M. McMeechan, J. Conder, ENVIRON International Corporation. The tissue residue approach seeks to use the concentration of a contaminant in tissue as a dose metric for evaluating toxicity and can be used in ecological risk assessment to evaluate whether contaminated sediment has the potential to cause harm to benthic invertebrates. Although concentrations of mercury in benthic invertebrate tissue associated with toxic effects from such sources as the United States Army Corps of Engineers (USACE) Environmental Residue Effects Database (ERED), reported values should be evaluated carefully to ensure relevance and applicability in environmental decision making. Publicly available information on tissue effect concentrations for total mercury (inorganic plus methylmercury) in both freshwater and saltwater benthic invertebrates were reviewed to establish a compilation of values useful as benchmarks of population-level effects in benthic invertebrates exposed to sediment containing mercury. Multiple lethal and sublethal (growth, development, behavior, and physiology) effects were considered. Tissue effect concentrations were selected with a preference for controlled, single-contaminant exposure studies with a dose-response relationship with a range well defined between No Observed Effect Doses (NOEDs) and Lowest Observed Effect Doses (LOEDs). Of the nineteen total mercury effects tissue effect concentration values reported, nine total mercury effects tissue effect concentration values ranging from 0.19 mg/kg, ww to 40 mg/kg, ww were found to be most relevant for characterizing mercury effects on a tissue concentration basis. The most common issues leading to exclusion of tissue effect concentrations from this compilation include: presence of multiple stressors during exposure, lack of a definitive demonstration of ecologically-relevant effects, and averaging values for multiple species. A study-by-study evaluation will be presented. Although tissue effect concentration data can be tabulated in a matter of seconds (e.g., via ERED), this review reinforces the need to carefully evaluate available tissue effect data for relevance in regards to the desired decision-making application.

**RP070 Assessing the Effects of Dietary Selenium on Survival and Reproduction of *Ceriodaphnia dubia*** M. Jatar, Univ of Ottawa; C.

Rickwood, Natural Resources Canada, CANMET MMSL, Univ of Saskatchewan, Dept of Toxicology; M. Pawlak, CANMET MMSL. Water Quality guidelines for selenium are not adequate in predicting toxicity to aquatic organisms, as the primary route of exposure is dietary. Revision of guidelines to encompass a fish tissue-based criteria are currently under way in both the US and Canada. While the primary focus of research examining dietary-Se exposures has been conducted with fish, very little information exists on dietary-Se exposures on invertebrates. In response to this knowledge gap, our objective was to elucidate the effects of dietary-Se on aquatic invertebrates. Specifically, the effects of water and food-borne selenium exposure on survival and reproduction of *Ceriodaphnia dubia* were compared. The recommended Environment Canada protocol (EPS 1/RM/21) was modified to incorporate selenium contaminated algae as an alternative food source. Exposures were conducted with both selenate and selenite over a 7-day period; survival and reproductive endpoints were measured. Exposure concentrations ranged from 1.25 to 40  $\mu$ g selenium/g algae. Results allow us to address the significance of dietary-Se on invertebrates and assess the protectiveness of current water quality guidelines. In addition, the modification of a standard sub-lethal toxicity test provided a simple method to assess dietary-exposure of contaminants to a commonly used invertebrate species.

**RP071 Assessing the Effects of Dietary Selenium on Survival and Reproduction of *Ceriodaphnia dubia*** M. Jatar, First Institute of Oceanography, State Oceanic Administration, Univ of Ottawa; C. Rickwood, Natural Resources Canada, CANMET MMSL, Univ of Saskatchewan, Dept of Toxicology; M. Pawlak, CANMET-MMSL. Water Quality guidelines for selenium are not adequate in predicting toxicity to aquatic organisms, as the primary route of exposure is dietary. Revision of guidelines to encompass a fish tissue-based criteria are currently under way in both the US and Canada. While the primary focus of research examining dietary-Se exposures has been conducted with fish, very little information exists on dietary-Se exposures on invertebrates. In response to this knowledge gap, our objective was to elucidate the effects of dietary-Se on aquatic invertebrates. Specifically, the effects of water and food-borne selenium exposure on survival and reproduction of *Ceriodaphnia dubia* were compared. The recommended Environment Canada protocol (EPS 1/RM/21) was modified to incorporate selenium contaminated algae as an alternative food source. Exposures were conducted with both selenate and selenite over a 7-day period; survival and reproductive endpoints were measured. Exposure concentrations ranged from 1.25 to 40  $\mu$ g selenium/g algae. Results allow us to address the significance of dietary-Se on invertebrates and assess the protectiveness of current water quality guidelines. In addition, the modification of a standard sub-lethal toxicity test provided a simple method to assess dietary-exposure of contaminants to a commonly used invertebrate species.

**RP072 A Study of Mercury, Methylmercury and Selenium Concentrations and Nitrogen Stable Isotopes in a Marine Tropical Food Chain** H.A. Kehrig, Universidade Federal do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; T.G. Seixas, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; A.M. Di Benedetto, C.M. Souza, C.E. Rezende, Universidade Estadual do Norte Fluminense, Laboratorio de Ciências Ambientais; O. Malm, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho. Trophic transfer of trace elements along marine food chains has been recognized as an important process influencing metal and metalloid bioaccumulation. Mercury (Hg), methylmercury (MeHg) and selenium (Se) concentrations and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope were assessed in two size classes of plankton, two top-predators, *Sotalia guianensis* (cetacean) and *Trichiurus lepturus* (fish), and their representative prey species (fish, cephalopod and crustacean species), which are distributed in different habitats along the water column of the northern Rio de Janeiro coast. This area in southern Brazilian coast belongs to a faunistic transition zone. The stable isotope was used to assess the modes of MeHg and Se bioaccumulation and biomagnification along the food chain. Hg and MeHg in the cetacean muscle were 2.5 times higher than those found in its preferential prey, *T. lepturus*. *T. lepturus* presented higher muscular Se concentrations than those found in cetacean (mean: 1.0 and 0.6  $\mu\text{g}\cdot\text{g}^{-1}$ , respectively). Cetacean presented the highest Hg and MeHg concentrations, followed by the benthic-feeder and planktivorous fishes, whereas the benthic-feeder fish showed the highest Se concentrations. The lowest Hg, MeHg and Se concentrations were found in planktonic organisms. This fact suggests that MeHg was transferred among the trophic levels from the lower trophic level to the top-level predators. Se showed



some evidence of trophic transfer between lower trophic levels. *S. guianensis* presented lighter  $\delta^{15}\text{N}$  (12.2‰) than their prey species (mean: 13.3‰), and this fact indicates that in our study the trophic position of this top-predator was not clearly related to muscular  $\delta^{15}\text{N}$ . The concentrations of Hg, MeHg, Se and  $\delta^{15}\text{N}$  increased successively with increasing trophic levels. The log HgX $\delta^{15}\text{N}$  and log MeHgX $\delta^{15}\text{N}$  regression slopes (0.26 and 0.31, respectively) indicated that this food chain presented elevated biomagnification rates that can be related to the environmental water quality. However, the log SeX $\delta^{15}\text{N}$  regression slope (0.12) indicated that this food chain presented a low biomagnification power for selenium. Hg and MeHg are biomagnified through the food chain while selenium does not present the same behavior. Se is biomagnified mainly in the lower and intermediate trophic levels.

**RP073 Mercury and Selenium in a Voracious Predator Fish, *Trichiurus lepturus* (Linnaeus, 1758), from Two Tropical Brazilian Areas** T.G. Seixas, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; I. Moreira, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Chemistry; O. Malm, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; H.A. Kehrig, Universidade Federal do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho. Mercury (Hg) and selenium (Se) were determined in the muscle tissue of cutlassfish (*Trichiurus lepturus*) caught by trawl nets or in gillnet fisheries in two different areas along the Rio de Janeiro coast, Brazil: Ilha Grande Bay (IGB) and Buzios coast (BC). IGB presents an oligotrophic environment and BC is an upwelling area. These areas presented different degrees of trace elements inputs and are subject to different environmental impacts. The diet of *T. lepturus* can be changed during the ontogeny, in which the specimens can be grouped by size (total length – TL). The specimens from BC were taken with three different ontogenic stages: large adults (> 100 cm – feed on pelagic and demersal fishes, including co-specifics), small adults (> 70-100 cm – feed on pelagic macrozooplankton, mainly juveniles fishes) and sub-adult (30 to 70 cm – feed on pelagic macrozooplankton, mainly euphausiids and juveniles fishes). The specimens from IGB were taken with two ontogenic stages: large and small adults. No significant difference ( $p > 0.05$ ) in total lengths was found between the specimens from the two sampling areas, suggesting that both groups are equally represented in the sample and making it possible to directly compare the concentrations of Hg and Se between them. Regional differences in the concentrations of these elements were observed in *T. lepturus*. The specimens collected at BC had muscular Hg concentrations about three times higher than those found in the specimens from IGB (mean: 1.07  $\mu\text{gHg g}^{-1}$  d.w. and 0.35  $\mu\text{gHg g}^{-1}$  d.w., respectively). However, the concentrations of Se did not differ between the sites, 1.01  $\mu\text{gSe g}^{-1}$  d.w. in BC and 0.96  $\mu\text{gSe g}^{-1}$  d.w. in IGB. These patterns were also observed for the same ontogenic stages. In both areas, a significant difference was found between muscular Hg and the ontogenic stage, i. e., the biggest specimens presented the highest Hg concentrations. This pattern was not observed for Se in both areas. The differences found between Hg in *T. lepturus* from both areas were probably due to the preferential prey of each ontogenic stage, bioavailability of elements in each marine environment, and environment variables (water temperature, net primary production). As a consequence, concentrations of trace elements in the tissues of this species can be considered to be a result of the surrounding environment.

**RP074 Lake Sedimentary Mercury Deposition and Fish Tissue Mercury Trends Associated with Recent Mercury Use and Emissions Changes in Massachusetts** M. Hutcheson, MA Dept of Environmental Protection, Office of Research & Standards; G.T. Wallace, Univ Of Massachusetts Boston, Dept of Earth, Environmental and Ocean Sciences; C.M. Smith, J. Rose, Massachusetts Dept of Environmental Protection, Office of Research and Standards; C. Batdorf, J. Sullivan, O. Pancorbo, Massachusetts Dept of Environmental Protection, Wall Experiment Station; C.R. West, Massachusetts Dept of Environmental Protection, Office of Research and Standards. The spatial pattern of historical (1800-2000) sedimentary mercury deposition trends inferred from analyses of 20 lake sediment cores from throughout Massachusetts will be presented in the context of historical mercury uses and atmospheric emissions in Massachusetts, with particular emphasis on trends in the last half of the twentieth century. In the last approximately 15 years there have been major state and regional regulatory efforts to reduce mercury uses and atmospheric emissions. Significant decreases occurred in total mercury concentrations in edible dorsal muscle of largemouth bass (*Micropterus salmoides*) and yellow perch (*Perca flavescens*) from these same

lakes during this period (1999-2009). Fish mercury levels generally reflected the higher levels of sedimentary mercury deposited during later twentieth century high mercury atmospheric release periods, and more recently reflect substantial decreases in mercury releases to the atmosphere. The results will emphasize the time taken for aquatic resources to respond to reductions in atmospheric inputs of mercury and present modeled projections of times for these fishery resources to achieve acceptable tissue levels of mercury to be safe for routine human consumption.

**RP075 Decreasing Aqueous Mercury Concentrations to Achieve Safe Levels in Fish: Examining the Water-Fish Relationship in 2 Point-Source Contaminated Streams** T. Mathews, Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge National Laboratory, Biological and Environmental Sciences Division; G. Southworth, Oak Ridge National Laboratory (retired), Environmental Sciences Division; M. Peterson, Oak Ridge National Laboratory; K. Roy, Oak Ridge National Laboratory, Environmental Sciences Division; R. Ketelle, Bechtel Jacobs. East Fork Poplar Creek (EFPC) and White Oak Creek (WOC) are two mercury (Hg)-contaminated streams located on the Dept of Energy's Oak Ridge Reservation in east Tennessee. East Fork Poplar Creek is the larger and more contaminated of the two, with average aqueous Hg concentrations currently exceeding those in reference streams by several hundred-fold. Over the past 20 years, extensive remedial actions have been taken that reduced aqueous Hg concentrations in EFPC by 85 %, but fish fillet concentrations have not responded to this decrease in aqueous Hg and remain above the Environmental Protection Agency's water quality criterion of 0.3 mg/kg for Hg in fish. The lack of correlation between aqueous and fish tissue Hg concentrations in this creek has led to questions regarding the usefulness of target aqueous Hg concentrations and strategies for future remediation efforts. White Oak Creek, located less than 10 km away from EFPC, has a similar contamination history but aqueous Hg concentrations in WOC are an order of magnitude lower than in EFPC. Despite the lower aqueous Hg concentrations, fish fillet concentrations in this creek have also been above the AWQC, making the most recent aqueous Hg target of 200 ng/L in EFPC seem unlikely to result in an effective decrease in fillet Hg concentrations. Recent monitoring efforts in WOC, however, have suggested that fish in this creek may be responding to lowered aqueous Hg concentrations. This new information could be useful to guide remedial actions in EFPC and in other point-source contaminated streams.

**RP076 Mercury in Demersal and Pelagic Fish Species from Ilha Grande Bay, Brazil** T.G. Seixas, I.A. Santos, L.A. Cassimiro, T.A. Cordeiro, O. Malm, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho; I. Moreira, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Chemistry; H.A. Kehrig, Universidade Federal do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho. Ilha Grande Bay (IGB; 22°S, 44°W) in Rio de Janeiro State has been subject of few mercury contamination studies. Mercury (Hg) is an exogenous and harmful metal, which accumulates in the tissues of aquatic organisms (such as fish) as they grow. It is the only trace element which biomagnifies through all level of the aquatic food web. This study assessed the concentration of Hg in the muscle tissue of seven demersal fish species (*Eugerres brasiliensis* – Brazilian mojarra, N= 17; *Cynoscion jamaicensis* – Jamaica weakfish, N=12; *Prionotus punctatus* – Bluewing searobin, N=9; *Diplacrum radiale* – Pond perch, N=7; *Polydactylus virginicus* – Barbu, N=11; *Citharichthys spilopterus* – Bay whiff, N=10 and *Micropogonias furnieri* – Atlantic croaker, N=19) taken accidentally by trawl nets, during commercial captures of shrimp (*Litopenaeus setiferus*) inside of IGB and also in a demersal-pelagic fish species (*Trichiurus lepturus* – Cutlassfish, N=21) that represent the voracious predator of this food web. These fish species has different feeding habit and some of them have no commercial value, but are consumed by local population. Hg concentrations were determined by CVAAS, using  $\text{NaBH}_4$  as a reducing agent. The more voracious demersal fish species, Brazilian mojarra and Jamaica weakfish, presented the highest Hg concentrations (0.15 and 0.12  $\mu\text{g g}^{-1}$  w.w., respectively). While, the lowest Hg was found in less voracious demersal fish species: Pond perch (0.08  $\mu\text{g g}^{-1}$  w.w.) > Barbu (0.06  $\mu\text{g g}^{-1}$  w.w.) > Atlantic croaker (0.05  $\mu\text{g g}^{-1}$  w.w.) > Bluewing searobin (0.04  $\mu\text{g g}^{-1}$  w.w.) > Bay whiff (0.03  $\mu\text{g g}^{-1}$  w.w.). The voracious predator (Cutlassfish) that feeds on the water column, showed lower Hg (0.05  $\mu\text{g g}^{-1}$  w.w.) than the more voracious demersal fish species, indicating that probably the sediments from this region are richer in Hg. The Cutlassfish also presented higher Hg concentration than its preferential prey (Atlantic croaker),

indicating that there is a positive transference of Hg from prey to predator. According to the results, probably, Hg has been suffering the biomagnification process in this web. However, more aquatic species must be studied to understand the behavior of this element in this environment. Probably, the differences in Hg among fish species can be related to the predominant food items for each species as well as the bioavailability of Hg in the marine environment.

**RP077 Arsenic Bioaccumulation in a Marine Juvenile Fish *Terapon jarbua*** Z. Wei, H. Liangmin, South China Sea Institute of Oceanology, Chinese Academy of Sciences; W. Wenxiong, Hong Kong Univ of Science and Technology. Arsenic (As) is a ubiquitous toxic metalloid causing a widespread public concern. Recent measurements indicated that marine fish in China can be seriously contaminated with As, but the biokinetics and bioaccumulation pathway of As in fish remains less well studied. In this study, we employed a radiotracer technique to quantify the dissolved uptake, dietary assimilation and subsequent efflux of As(V) in a marine predatory fish, *Terapon jarbua*. Dissolved uptake of As showed a linear pattern over a range of dissolved concentrations from 0.5 to 50  $\mu\text{g L}^{-1}$ , with a corresponding uptake rate constant of 0.0012 to 0.0017  $\text{L g}^{-1} \text{d}^{-1}$ . The dietary As assimilation efficiencies (AEs) were only 3.1-7.4% when the fish fed on different preys (copepods, clams, prey fish, and artificial diet), and were much lower than its partitioning in the trophically available metal fraction (TAM) in the prey clam and fish. The dietary AEs were independent of the As(V) concentrations in the artificial diets. The efflux rate constant of As in fish following the dietary exposure was 0.06  $\text{d}^{-1}$ . Modeling calculations showed that dietary uptake could be the primary route for As bioaccumulation in fish, and the corresponding contribution of waterborne and dietary uptake was related to the bioconcentration factor (BCF) of the prey and ingestion rate of fish. Our study showed that As(V) had a low bioavailability to marine fish.

**RP078 Investigation of Toxicity of Multi-wall Carbon Nanotube in *Caenorhabditis elegans* Using Functional Toxicogenomics Approach** J. Roh, Univ of Seoul; M. Bruno, Y. Ge, USEPA; J. Yi, Seoul National Univ; J. Choi, Univ of Seoul, School of Environmental Engineering. Carbon nanotube (CNT) has a wide application in industrial and biomedical fields, which could end up their occurrence in the environment. Associated hazard and risk should, therefore, be investigated, before their widespread use. In this study, toxicity of multi wall carbon nanotube (MWCNT) was investigated in the soil nematode, *Caenorhabditis elegans* using integrated systems toxicology approaches. Toxicity of MWCNT was first tested using survival and reproduction. Toxic mechanism of MWCNT was investigated using microarray based gene expression analysis. Integrated bioinformatics tools were applied to gain an insight of worm's global response to MWCNT exposure and quantitative PCR analysis was followed on the selected genes. Functional analysis was subsequently conducted, analyzing gene expression data coupled with phenotypic measurements. Overall results indicate that a combination of multiple pathways, including oxidative stress and inflammation, may be required toxicity of MWCNT in *C.elegans*, which was frequently observed in mammalian in vitro and in vivo models. It is important to note that negative or insignificant results at the organism level should not be considered as MWCNT being "safe" nanomaterials, as significant alteration on the gene expression was revealed by microarray and subsequent PCR and functional analysis, which may eventually be manifested at higher level after long term exposure. Results also suggest that system-based integrated toxicogenomic approach is particularly valuable to investigate toxicity of new chemicals of which mode of action is not fully understood or incompletely characterized, such as, MWCNT. This work was supported by the National Institute of Environmental Research, the Ministry of Environment of Korea and the Ministry of Education, Science and Technology of Korea [2010-0016195].

**RP081 Comparison of Carbon Nanotube and Asbestos Exposure on the Gill and Liver Histology of Fathead Minnows** A. Edgington, Clemson Univ, Biological Science and CU-ENTOX, Clemson Institute of Environmental Toxicology; K. Donohue, J. Laird, D.R. Johnson, J. Stanley, J.A. Steevens, US Army Engineer Research and Development Center, Environmental Laboratory; S.J. Klaine, Clemson Institute of Environmental Toxicology, Clemson Univ. Carbon nanotubes (CNTs) are graphene cylinders with a long common axis resulting in a fiber-like characteristic. Carbon nanotubes have unique characteristics that include durability and

high aspect ratio (ratio of length and width) that has been compared to that of asbestos fibers. These similarities between asbestos and CNTs have raised concerns about potential toxicity. Many inhalation studies have shown the potential for asbestos to cause physiological effects to lung tissues often resulting in carcinomas. But little research has been done on aquatic exposures of asbestos to fish species. The objectives of this project were to compare the physiological effects of CNT and asbestos exposure to *Pimephales promelas*, commonly called the Fathead Minnow (FHM). Comparisons were made using two forms of CNTs, single-walled and multi-walled nanotubes, and two forms of asbestos, crocidolite and chrysotile. Adult FHM were exposed to 0.125, 0.25, and 0.5  $\text{mg/L}$  of suspended CNT or asbestos in a solution of Suwanee River water and tanks were renewed daily. Organisms were sacrificed at days 0, 7, and 14 and gills and livers were collected and fixed in gluteraldehyde. Samples were sectioned, stained and examined on a microscope for physiological abnormalities. Samples were also fixed and sectioned for transmission electron microscopy (TEM) coupled with electron emission loss spectroscopy (EELS) to determine CNT absorption through the gill. Carbon nanotube and asbestos particles were characterized using TEM, zeta potential, and dynamic light scattering to determine particle characteristic effects on tissue histology and uptake.

**RP082 Influence of Media Chemical Properties on Aggregation Behavior of Copper Nanoparticles** N.B. Saleh, Univ of South Carolina, Civil and Environmental Engineering; V. Shah, Dowling College; A. Afroz, Univ of South Carolina, Civil and Environmental Engineering; F. Rispoli, Dowling College, Dept of Mathematics. Unique physical, chemical, and biological properties of copper nanoparticles (CuNPs) have generated considerable interest in environmental and medical fields. This study focuses on the fundamental mechanism of aggregation of CuNPs in influence of nine different chemical parameters. Parameters selected for the study include: pH, concentration of mono- and di-valent cations ( $\text{K}^+$ ,  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ), natural organic matter, bovine serum albumin, and sugars (alginate and fructose). Aggregation behavior was measured using time-resolved dynamic light scattering. Aggregation structure is further studied in suspension by cryo transmission electron microscopy. Furthermore, temperature dependent size measurement is performed to probe the secondary minima of these colloids to study reversible attachment. The findings from this study can help carefully probe CuNP aggregation mechanism in diffusion limited aggregation regime and will shed light onto the role of secondary minima in reversible attachment of such particles.

**RP083 Toxicity Effect of Ferrous Sulfide and Zero Valent Iron Nanoparticles on *Escherichia coli* Growth Under Anaerobic Conditions** T.M. Olson, M.R. Higgins, Univ of Michigan, Dept of Civil and Environmental Engineering. In situ, passive technologies for remediating contaminated groundwater offer potential economic and environmental advantages over ex situ, active extraction methods. Most in situ technologies involve emplacing reactive materials in the path of a contaminant plume with the goal of transforming or sequestering contaminants in the reactive zone. Approaches to effectively create the reactive zones, known as permeable reactive barriers (PRBs), have typically involved the use of reduced iron materials, such as zero valent iron (ZVI), but more recently other iron mineral nanoparticles, such as ferrous sulfide, have been investigated. Ferrous sulfide readily forms as mackinawite nanoparticles at ambient temperature and offers similarly reactive capacity as ZVI. Although incorporating nanoparticles into PRBs is potentially beneficial due to their high reactive surface area, their release risk and toxicity have not been extensively tested. In this study, the toxicity of nano FeS particles to model bacteria, *Escherichia coli*, was evaluated in terms of their effect on growth rates in anaerobic cultures. The experiments demonstrate that *E. coli* growth is inhibited by the presence of FeS, however, the inhibition is not significantly different than that observed in particle-free sodium sulfide solutions at FeS-sulfide saturation concentrations. Growth inhibition, therefore, was not directly caused by the nano-FeS itself. The toxicity effects of nano-ZVI and FeS will be compared and implications for the use of these materials in PRBs will be discussed.

**RP084 Multi-linear Regression Methods to Develop an Integrated Addition Model for Predicting Mixture Toxicity** J. Kim, KIST Europe, Knowledge Research Group, Univ of Koblenz-Landau, Institute of Environmental Sciences; S. Kim, KIST Europe, Chemical Management Lab.; G.E. Schaumann, Univ of Koblenz-Landau, Institute of Environmental Sciences. Living organisms are actually exposed to chemical mixtures. However,

chemical risk assessment frequently places less focus on chemical mixtures than individual substances. In addition, recent studies on mixture toxicity shows that single substances at levels below no-observed-effect concentrations (NOECs) may cause toxicity due to the mixture toxicity among substances. For human health and the environments, the mixture toxicity thus needs to be considered in mixtures risk assessment. The concepts of additive toxicity, concentration addition (CA) and independent action (IA) models, are often applied to predict the mixture toxicity of similarly- and dissimilarly acting chemicals, respectively. However, living organisms and the environments are exposed to both types of chemicals at the same time and space. Hence, it needs to develop an integrated model to predict mixture toxicity from different chemicals practically. The objective of this study is to develop an integrated addition model based on the multi-linear regression for estimating mixture toxicity in the environment risk assessment. In this study, different multi-linear regression (MLR) methods are employed to integrate the CA and IA models, and then evaluated to select the best MLR method. The results show that the prediction capability of the integrated addition model based on MLRs is outstanding as compared with the conventional CA and IA models. Ridge- and Partial Least Squares-based regression methods outperform the other MLR methods.

**RP085 A Watershed-scale Ecosystem Services Life Cycle Impact Assessment Approach to Water Risk Assessment** M. Matlock, Univ of Arkansas, Centre for Agricultural and Rural Sustainability; G. Thoma, Univ of Arkansas, Dept of Chemical Engineering. Water resources are a global concern but a local issue. Measuring the impact associated with manufacturing consumer package goods at a specific site is difficult. Current impact assessment methods are primarily geared towards agricultural water use and lack the ability to effectively measure the potential risks or impacts associated with industrial freshwater use and depletions at the site level. We will present an approach to water resources risk assessment based on a watershed-scale site life cycle impact assessment framework. Site-specific local water scarcity were evaluated at watershed scale and the percent sector (e.g., industrial, agricultural, domestic) water usage were determined for 150 manufacturing facilities in the food, beverage and agriculture sector in order to analyze the magnitude that each sector contributes to local water consumption. This analysis was the baseline for the Level 1 Impact Assessment. The Global Water Tool (GWT) developed by the World Business Council was used to evaluate the total renewable water resources per person and the percent of the population with access to improved water and sanitation at each site. The GWT data was linked with Pfister et al.'s Water Scarcity Index (WSI) to determine per capita annual renewable water supply, projected per capita annual renewable water supply for 2025, the mean annual relative water stress index, and Pfister's WSI. The Level 2 Water Impact Assessment was developed from data provided by companies participating in the prototype project. A water use inventory for each category of facility was developed and distributed to participating companies, and data were analyzed on a site basis. Parameters that were analyzed include water use impact on scarcity, water use impact on water quality, and water use impact on ecosystem services. Facility discharge water quality parameters were evaluated for potential risk to ecosystem services and human health based on the waste load in the wastewater discharge. The wastewater discharge was characterized from survey information. The effectiveness at measuring risk at the facility level was evaluated by comparing sites globally. Areas of evaluation included potential damage to human health, ecosystem services, and biodiversity.

**RP086 Addressing Risk and Uncertainty in USACE Ecosystem Restoration Projects** B.C. Suedel, US Army Engineer Research and Development Center, CEERD-EP-R, US Army Engineer Research and Development Center, Environmental Laboratory, Waterways Experiment Station EP-R; J.T. Vogel, US Army Engineer Research and Development Center; M. Convertino, Univ of Florida, Agricultural and Biological Engineering; I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center, US Army Engineer Research and Development Center; J. Valverde, T.S. Bridges, US Army Engineer Research and Development Center. Several factors constrain effective use of risk and uncertainty tools and techniques in ecosystem restoration. Even though risk is a crucial driver in assessing effectiveness of ecological restoration and risk assessment has evolved both as analytical method and regulatory tool, its application to ecosystem restoration projects has not been formalized or fully explored. Further, tools and techniques for addressing uncertainty developed for other disciplines have been applied to few ecosystem restoration projects. The purpose of this work

was to frame and document the application of risk management concepts and methods for ecosystem restoration as practiced within the USACE. Case study examples of recent USACE district projects employing risk and uncertainty best practices for ecosystem restoration projects were identified and described, including those addressing the Sensitivity of the Barataria Basin Barrier Shoreline Wetland Value Assessment Model, the Upper Mississippi River System Navigation and Ecosystem Sustainability Program, and Quantifying and Communicating Model Uncertainty for Decision Making in the Florida Everglades. Tools and techniques representing the state of the science for addressing risk and uncertainty were then identified that could further advance the application of risk and uncertainty to ecosystem restoration. A hypothetical case study developed for the work will also be presented, illustrating how risk management and uncertainty tools and techniques can be integrated into the USACE ecosystem restoration projects.

**RP087 An Ecosystem Services Framework in Practice: An Assessment of Fruit Production in Southern Europe** G. Reub, ENVIRON International Corp, Ecology and Sediment Management; S. Deacon, ENVIRON UK Ltd; G. Greene, ENVIRON International Corporation, Ecology & Sediment Mngmt; S. Norman, Dow Agrosiences. In this pioneering proof-of-concept study an ecosystem services framework was developed and applied to a case study in support of the re-registration of a well-known insecticide for use in Southern European countries where citrus growing is particularly important both economically and culturally to the communities in these regions. The study focusses on Southern Spain where generations of farmers have grown citrus fruits. The re-registration is in compliance with the European Plant Protection Products Directive. The European Food Safety Authority (EFSA) recently developed and published a framework for developing specific protection goals for the environmental risk assessment of pesticides that includes an ecosystem services approach. This ecosystem services concept takes a holistic view of environmental, social and economic factors. The ecosystem services approach identifies and values the primary environmental services that a land cover type may provide given different land uses and actions. The type, quantity, and quality of environmental services provided are influenced by management actions on the land. A systematic evaluation of such changes in service flows is provided to allow for consistent comparisons across two alternatives that examine management actions and include the use of certain pesticides among other activities that affect ecosystem services. The framework allows for the formal quantification (semi-quantification) of ecosystem service values associated with different land cover types and the anthropogenic influences within each cover type. The approach relies on gathering existing data and development of an analytical framework. The approach governs the collection and analysis of data on a detailed land cover type scale, establishing existing capacities of ecosystem services (baseline conditions). The results produce a composite measurement of baseline conditions expressed in a standard metric or "currency" (e.g., service hectares) allowing ecosystem service levels to be compared. Results are then estimated for conditions after a change in a management action (such as the application of a pesticide) and the net ecosystem service gains and losses between scenarios or condition levels may be compared. Ecosystem services include those related to physical, chemical, biological, and human use and non-use categories.

**RP088 Agrochemical Mitigation of Three Aquatic Macrophytes: Implications for Ecosystem Services** H.L. Tyler, USDA ARS National Sedimentation Laboratory; M.T. Moore, USDA-ARS, National Sedimentation Laboratory, USDA-ARS National Sedimentation Laboratory, National Sedimentation Laboratory; M.A. Locke, USDA ARS National Sedimentation Laboratory. Agricultural runoff containing nitrogen and phosphorus is a major contributor to eutrophication in aquatic systems. Vegetated drainage ditches lining agricultural fields have been investigated for their potential to mitigate runoff, acting similarly to a wetland as they filter contaminants. The efficiency of three aquatic macrophytes, cutgrass (*Leersia oryzoides*), cat-tails (*Typha latifolia*), and bur-reed (*Sparganium americanum*), to mitigate ammonia, nitrate, and phosphate from water was investigated. Replicate mesocosms of each plant species were exposed to water enriched with ammonia and nitrate for 6 h and flushed with clean water for an additional 6 h to simulate storm events. After termination of the simulated runoff, all vegetated treatments lowered total Kjeldahl nitrogen loads exiting mesocosms by greater than 50%, significantly more than unvegetated controls. *Leersia* and *Typha* were more efficient at lowering dissolved nitrogen, lowering ammonia 42 and 59% and nitrate by 67 and 64%, respectively. *Leersia*



and Typha decreased the loads of dissolved phosphate exiting mesocosms by 50% while Sparganium only decreased dissolved phosphate by 15%. All treatments decreased ammonia and nitrate concentrations within mesocosms by more than 86% after one week, though Typha and Leersia acted more rapidly. In determining the agrochemical mitigation efficiency of different plant species, vegetation in drainage ditches can be better maintained for optimal remediation of agricultural runoff.

**RP089 Ecosystem Restoration: Development of Project Level Objectives and Metrics** J.T. Vogel II, K. McKay, B. Suedel, M. Convertino, I. Linkov, US Army Corps of Engineers, Engineer Research and Development Center. Accounting for the benefits of ecosystem restoration projects requires defining multiple metrics corresponding to ecosystem structure, function, and processes. Metrics measure progress toward goals and objectives, raise awareness and understanding, and support restoration decision making, especially in cases where uncertainty is prevalent. Multiple metrics may be defined which have a varying degree of utility for restoration managers. This presentation will describe the underlying scientific principles and best practices for the development and application of metrics for environmental benefits analysis. We will also present decision analysis techniques as a means for integrating large amounts of varied types of information. This optimizes decision-making processes as they relate to developing objectives and metrics for ecosystem restoration projects. Decision analysis allows for the investigation of the costs and benefits of different decisions available and the assessment the trade-offs between restoration options. These principles and practices will span three sub-topics related to metrics, namely: (1) identification of metrics corresponding to project objectives; (2) analytical techniques for comparing and trading-off metrics; and (3) application of metrics within a restoration decision hierarchy, from the programmatic to the regional level. The regulatory realities in which restoration projects must take place (i.e., NEPA and Clean Water Act) are also addressed.

**RP090 A Compendium of Transcriptomic Effects of Endocrine Disrupting Chemicals on the Fathead Minnow Ovary** T. Habib, BTS; L. Escalon, US Army, Engineer Research & Development Center; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; D. Bencic, USEPA, Office of Research and Development, USEPA; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division; N. Garcia-Reyero, Jackson State Univ. Understanding potential hazards of chemicals released into the environment is challenging not only due to the large and growing number of chemicals and materials that need to be screened, but also to the bioavailability, exposure conditions, and species differences among others. Examining effects on critical pathways resulting in adverse outcomes of interest such as reduced reproduction provides a broader measure of the potential hazards of a chemical. Effective mining of complex relationships, effects and potential impacts can be gained through meta-network analyses derived from expression data. The Hypothalamus-Pituitary-Gonadal (HPG) Axis is an evolutionarily conserved endocrine pathway principally responsible for the control of reproduction. Measuring impacts of chemicals on the HPG axis provides a functional measure of the chemical's reproductive hazards. Therefore, analysis of steroidogenesis and the biological elements controlling it can be used to understand chemical effects and key events leading to adverse reproductive outcomes. Here, we used network inference approach to investigate the impacts of chemicals on steroidogenesis. Fathead minnows (*Pimephales promelas*) were exposed to known endocrine disruptors and impacts on steroidogenesis were examined through network analysis of a large compendium of ovarian gene expression and hormone data sets. The compendium contained 1,472 microarrays, with data from twenty-three different in vivo exposure experiments encompassing 13 different chemicals, 1 complex mixture, 5 ovary stages, multiple time points, and multiple chemicals doses. Transcriptional networks were obtained from two different sources: 1) all 23 exposures and 2) seven individual chemicals (fadrozole, flutamide, prochloraz, trenbolone, vinclozolin, trilostane, and ketoconazole). Gene networks inferred by both approaches were consistent with minor differences and both were able to detect important interactions from genes involved in reproduction and development pathways (such as estrogen and progesterone signaling). Common interactions between the combined and individual networks can offer insights

about the common mechanism whereas the difference in the interactions can provide the specific chemical effect.

**RP091 Analysis of Gene Expression in Endosulfan Exposed *H. americanus* Larvae Using an Oligonucleotide Microarray** M. Bauer, Univ of Prince Edward Island, Dept of Pathology & Microbiology, Atlantic Veterinary College, Univ of Prince Edward Island, Environment Canada; S. Greenwood, Univ of Prince Edward Island, Dept of Biomedical Sciences, Atlantic Veterinary College; P. Jackman, Environment Canada, Toxicology Laboratory. Agriculture, aquaculture and fisheries are essential industries in Atlantic Canada that must be sustained while also implementing measures to reduce their environmental impact. The use of pesticides in agriculture and aquaculture are a cause for concern in regards to the chronic and acute effects on non-target organisms. Endosulfan is a moult inhibiting organochlorine pesticide that is sprayed on potato fields on Prince Edward Island to eliminate insect pests. Following rain events, endosulfan has been found in nearby marine and aquatic environments as a result of soil run-off and is hypothesized to have negative effects on marine crustaceans. The current research project focuses on the effect of endosulfan on the American lobster (*Homarus americanus*), an important commercial resource. Stage III larvae were chronically exposed to environmentally relevant concentrations of endosulfan (0.01, 0.03, 0.1 0.3 & 1.0 ug/L) and were found to exhibit developmental delays in development to stage IV and mortality. Microarray analysis was used to detect changes in genes expression between each concentration and to identify pathways being affected by endosulfan exposure. The results of this study will identify the effects of endosulfan on lobster larvae development, survival and the potential impact on the lobster population.

**RP093 Polyploidy in Leukemic *Mytilus* sp. Haemolymph: A Biomarker for Population Health** E. Vassilenko, Univ of British Columbia, Chemical and Biological Engineering; S. Baldwin, Chemical & Biological Engineering, Univ of British Columbia. Mussels have been used for environmental monitoring since the 1970's. Leukemia or haemic neoplasia (HN) is a sub-lethal endpoint that can be used for assessment of mussel population health. HN is associated with cell morphology alterations and with abnormal ploidy in haemocytes. We used flow cytometry (FC) to analyze haemocyte ploidy and haemocytology to assess percentage of neoplastic-looking cells in the haemolymph of two species, *M. trossulus* and *M. edulis*. We analyzed samples of mussel populations collected during an exposure experiment conducted in 2010 in the Vancouver area (BC, Canada). The experiment had a 2x2 factorial design with two control samples of non-exposed populations at the beginning and at the end of the experiment. *M. trossulus* were collected from two different beach locations and *M. edulis* were purchased from a farm in April. Both species were exposed on submerged frames from April to October at two sites, one urban and the other remote. In October additional sampling of beach populations were conducted. Using a new approach to FC ploidy analysis and classification of the detected ploidy patterns, our results show that progression of HN is different in natural beach populations and in populations on the submerged frames. As well we show that the prevalence of late-stage HN and the amount of animals with abnormal ploidy were higher for the urban exposure site compared with the remote site. We suggest that the ploidy analysis might be considered as a sensitive marker for monitoring of mussel population health.

**RP094 The Copepod *Calanus finmarchicus*: A Promising Cold Water Model Species for Ecotoxicology and Environmental Genomics Related to Oil Exposure** B. Hansen, SINTEF Materials & Chemistry, Marine Environmental Technology; D. Altin, BioTriX; A.J. Olsen, Dept of Biology, NTNU; T. Nordtug, SINTEF Materials and Chemistry; K. Degnes, SINTEF Materials and Chemistry, Biotechnology; T.R. Storseth, SINTEF Fisheries and Aquaculture; H. Sletta, SINTEF Materials and Chemistry, Biotechnology; K.E. Tollefsen, NIVA, Ecotoxicology & Risk Assessment. In recent years there has been an increasing emphasis on the significance of invertebrates for assessing environmental impact of oil spills. In the event of accidental oil spills in sub-Arctic and Arctic marine environments planktonic organisms like copepods are realistic targets for oil toxicity based on their significant role in the food web, their abundance and their large lipid content. For many years the SINTEF/NTNU culture of *Calanus finmarchicus* has functioned as relevant model for standard ecotoxicity testing of North Atlantic crude oils, and a large database of ecotoxicity data exists for this species. Parameterized experimental data on the effects of oil (single oil

components, water soluble fraction of oil, and dispersed oil) on copepod survival and reproduction are used as input for development of numerical models for environmental risk and damage assessment. The accessibility of a continuous copepod culture provides homogeneous specimen in terms of developmental stage and lipid content. This, along with sophisticated experimental systems, is a major foundation for investigating effects of stressors on molecular systems. A 60K oligonucleotide microarray has been developed based on a combination of available NCBI expressed sequence tags (ESTs) and 260,000 ESTs sequenced by FLX454 technologies. Several methods, like <sup>1</sup>H-nuclear magnetic resonance and mass spectrometry, have also been developed and applied in order to investigate metabolic profiles and alterations. Together these complementary methods give valuable supporting information to well known fitness-related endpoints, as they contribute with modes of toxic action of stressors, and output data may also be used to determine effect limits. The presentation will include results from experiments aimed at investigating the effects of chemically and mechanically dispersed oil, their modes of toxic action, and proposed effect limits of toxicity based on fitness-related endpoints as well as molecular profiling.

**RP095 Atrazine Residue and Glutathione S-Transferase Response of Freshwater Mussel *Uniodora contradens* Living in Agricultural Catchments, Northern Thailand** T. Thitiphuree, Chulalongkorn Univ, Chulalongkorn Univ, Dept of Biology, Faculty of Science; J. Kitana, Chulalongkorn Univ, Dept of Biology, Faculty of Science; P. Varanusupakul, Chulalongkorn Univ, Dept of Chemistry; N. Kitana, Chulalongkorn Univ, Dept of Biology. Northern part of Thailand is known as an origin of several rivers and tributaries as well as a fertile area for agricultural activities. Seasonal cultivation in this area leads to widely uses of agrochemicals especially atrazine herbicide. To examine whether an intensive use of atrazine could lead to contamination in aquatic environment, the sediment and water were collected from agricultural catchment basin in Nan province, northern part of Thailand during 2010–2011 and subjected to analysis for atrazine by gas chromatography-mass spectrometry. The results showed that detectable levels of atrazine residue were found in water (maximum value 0.16 mg/L) and sediment (maximum value 0.23 mg/kg) of the catchment area. Since atrazine is also known to cause disruption of organ systems of animal, it is thus important to monitor its effects on representative species of aquatic animal. In this study, a freshwater mussel *Uniodora contradens* was used as a sentinel species for potential health effects of atrazine contamination in aquatic environment. Freshwater mussels were collected from the agricultural catchment area during July 2010 to June 2011. Mussels were subjected to tissue residue analysis for atrazine by high performance liquid chromatography and examine for specific activity of glutathione s-transferase (GST), a biotransformation enzyme, in their hepatopancreas by spectrophotometric assay. The results on seasonal profiles of atrazine residue and specific activity of GST will be presented. Association between environmental and tissue residues of atrazine and potential oxidative stress condition of the freshwater mussel will be discussed. The information from this study could be used as an early warning of health effects of low level atrazine contamination on freshwater animals and the potential link to other organism in the area including human.

**RP096 Evaluation of Algal Estrogen Exposure Scenario Focusing on Gengorobuna as a High-exposure Group** Y. Hida, The Univ of Shiga Prefecture, Ecosystem Studies, The Univ of Shiga Prefecture; Y. Fujimoto, K. Kitao, M. Nakamura, T. Shibata, H. OOkura, Y. Yamada, S. Kuribayashi, T. Kurata, The Univ of Shiga Prefecture. A class of chemicals called endocrine disruptors, which were pointed out in 1990s, still has been an “unknown” type of risk in the society. While clear cause-effect relationships about endocrine disruptors have not yet been established, in some countries, the concern that the uncertainty in their risk estimation may be excessive large results in the society’s decision-making to avoid them completely. Thus, if the current risk assessment methodology of endocrine disruptors is that its do not affect the society’s decision-making, efforts should be improved to reduce its uncertainty. In this sense, it is critical to fundamentally characterize endocrine-disrupting action itself using field data. Since 2004, we have shown that algae (phytoplankton), which are ubiquitous in the aquatic environment, have weak estrogenic activities, are taken up passively, and act on gonadal development by temporarily suppressing maturation and the seasonal maturation phase in round Crucian carp (Nigorobuna). In this study, we focused on Gengorobuna as a group that is highly exposed to algae among Crucian carp species, which are supposed to eat zooplankton or

aquatic animals generally. Gengorobuna is the only carp which are supposed to eat phytoplankton, and since it has almost twice the number of gill rakers as Nigorobuna, it is probably more affected by algae exposure. Therefore, by comparing gonadal development in these two carp, we can show the one example about the effects of algae on fish based on the actual amount of algae they are exposed to in nature. When 5-month-old Gengorobuna and Nigorobuna maintained under the same cultivation environment with excessive algae flourished were compared, serum vitellogenin (VTG) concentrations in Gengorobuna were lower in both sexes, and the gonadosomatic index (GSI) was skewed to low values, —particularly in males. Seasonal variations in GSI and VTG in mature Gengorobuna in Lake Biwa are nearly always lower than those in Nigorobuna throughout the year in both sexes. Furthermore, in breeding experiments with Gengorobuna exposed to Chlorella, which have estrogenic activity, gonadal maturation phase was preceded in a control group of males compared with that in an exposed group and lagged in females, and the difference was clearer in both sexes of Gengorobuna when compared with Nigorobuna. These results are consistent with the hypothetical scenario that fish are exposed to estrogenic substances through algae consumption in nature.

**RP097 Integrating Toxicity Test and Experimental Ecosystem Data to Better Define Aquatic Risks** D.R. Mount, US Environmental Protection Agency, ORD; R.J. Erickson, USEPA, Mid-Continent Ecology Division. Aquatic risk assessments based on species sensitivity distributions can be uncertain regarding the nature and magnitude of the effects being addressed. The use of inconsistent endpoints and the failure to consider complete effects/exposure relationships contribute to this uncertainty. What percentile is selected as a level of concern in the distribution also represents a major uncertainty. Better definition of risks can be achieved by more fully exploiting information from toxicity tests, using more consistent endpoints, and relating results from toxicity tests to effects in experimental or natural ecosystems. Using atrazine effects on aquatic plants and plant communities as an example, this talk will discuss an index for better describing the level of toxicity to an assemblage of species as a function of concentration and time, and how experimental ecosystem data can be used to support risk characterizations based on this index.

**RP098 Putting “Health” Back into Ecosystem Health Assessment** K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology. There are many different purposes to collecting information, and trying to merge predictive and retrospective frameworks is very difficult. A major challenge is that situations where toxicity is strong show positive results across a spectrum of responses, including laboratory and field approaches. Regardless of the assessment purpose, objectives, approach, design and analysis, indicators have to be practical, simple, and consistent, with a low type II error rate. Laboratory approaches sacrifice ecological relevance, while field approaches have to make compromises in terms of conflicting requirements for protection, detection, reversibility, causality and ecological relevance. Ecosystem health assessment has largely followed a human health medical paradigm, where reference collections seek to define the level of natural variability in terms of spatial and temporal fluctuations that can mask impacts. Historically, definitions of “normal” levels of responses are used to detect deviations from normal and the absence of “health”. There is perhaps a more useful paradigm, where the ability of organisms to integrate responses can be used holistically to evaluate performance. Under this effects-based paradigm, study designs seek out natural variability, and a system self-defines its level of health, thresholds of responses, and triggers to inform management decisions. Relationships also translate ecological information into currencies relevant for land use planning, natural resource management and impact mitigation. It represent a simpler site-specific approach that requires a commitment to baseline monitoring, consistency, and commitment to long term planning that is usually absent in current situations.

**RP099 Relationships Between Atrazine Contamination and Biologic Response of Paddy Crab, *Esanthelphusa nani*, in Agricultural Area of Nan Province, Thailand** R. Maneen, Chulalongkorn Univ; W. Khonsue, Chulalongkorn Univ, Dept of Biology; P. Varanusupakul, Chulalongkorn Univ, Dept of Chemistry; N. Kitana, Chulalongkorn Univ, Dept of Biology. A variety of herbicides has been used intensively in agricultural activities at Nan province, northern part of Thailand. Although adverse effects on non-target organisms of these herbicides, especially atrazine, have been reported,

information on exposure-response relationships of atrazine on freshwater invertebrate is still limited. Gas chromatography-mass spectrometry analysis for residue of atrazine in soil and water of paddy fields in Nan province showed that detectable level of atrazine (0.15 mg/L) can be found in water of the intensive herbicide utilization area. Therefore it is crucial to monitor atrazine contamination and potential health hazards to the non-target organisms living in the herbicide utilization area. A rice field crab ESAN-THELPHUSA NANI has been selected as a sentinel species because it lives in paddy fields with direct exposure to herbicide contaminated soil and water. In this study, crabs were field collected for one year from two study sites: a reference site where no herbicides were used and a contaminated site where herbicides were used intensively. Crabs were subjected to contaminant analysis for atrazine in tissue by HPLC technique and determination of specific activity of glutathione S-transferase (GST), a detoxify enzyme in hepatopancreas, by spectrophotometric assay. Seasonal change in levels of atrazine tissue residue and a biologic response of the crab to atrazine contamination in term of GST activity will be presented. Association between environmental and tissue residues of atrazine and potential oxidative stress condition of the freshwater crab will be discussed and used for assessing a potential impact of atrazine contamination on non-target organisms in agricultural environment.

**RP100 Modifying Foods and Feeding Regimes to Optimize the Performance of *Hyalella azteca* During Chronic Toxicity Tests** J.R. Hockett, T.L. Highland, D.J. Hoff, D.R. Mount, USEPA, Mid-Continent Ecology Division; T.J. Norberg-King, USEPA; T.R. Valenti, National Research Council, c/o USEPA. The amphipod *Hyalella azteca* is commonly used to assess the toxicity of sediments and waters. However, laboratories have reported varying success in maintaining healthy cultures and in obtaining consistent growth and reproduction (where applicable), especially during tests conducted for longer durations (i.e., 28 and 42 days). Even where control survival criteria are met during 10-d tests, poor growth and reproduction observed during longer tests under similar conditions may bring into question whether the results of shorter tests provide a reliable measure of toxicity. Some of the challenge to consistency in testing with *H. azteca* may stem from uncertainties regarding environmental conditions necessary for successful culture and testing of this species; an informal survey of laboratories indicated a wide variety of water types and feeding regimes in use. Until these uncertainties are resolved, it seems reasonable that laboratories could prove the adequacy of their test conditions for *H. azteca* by demonstrating good survival, growth, and reproduction in a 42-d control exposure using a neutral substrate, such as clean quartz sand. Previous work in our laboratory showed that 10-d amphipod growth could be markedly improved by amending Lake Superior water with 10 mg chloride/L (added as NaCl). As part of evaluating this water's suitability during 42-d tests as described above, we also included treatments using some different diets and ration sizes. Although the amended Lake Superior water did appear to support good organism performance, we found evidence that the standard feeding rate of 1 ml YCT/chamber/day (based on current EPA and ASTM guidance) was not sufficient to sustain optimal growth and reproduction for periods longer than the initial 10 days of the test. Following from this observation, additional studies were conducted to more thoroughly evaluate different foods, combinations of foods, feeding rates, and temporally increasing feeding rates (intended to account for the changes in organism mass over the course of the test). Detailed comparisons within and between experiments are discussed. While work to define a new feeding regime for use with *H. azteca* is not yet complete, we believe the results thus far strongly indicate that the standard feeding rate of 1 ml YCT/chamber/day should be increased in latter portions of 28-d or 42-d tests with *H. azteca*. This abstract does not necessarily reflect EPA policy.

**RP101 Well Past Time to Stop Using NOEL/LOELs** W.G. Landis, Institute of Environmental Toxicology, Western Washington Univ, Western Washington Univ, Institute of Environmental Toxicology, Western Washington Univ, Institute of Environmental Tox. & Chem.; P.M. Chapman, Golder Associates Ltd. In the popular song, *Hotel California*, by the band the Eagles, "they just can't kill the beast". That is exactly our situation – we (and many others) have not yet been able to kill the "beast" that comprises No Observed Effect Levels (NOELs) and Lowest Observed Effect Levels (LOELs). Given that the concentration-response curve is one of the founding paradigms of environmental toxicology it is absurd that hypothesis testing continues to be used to report toxicity. We submit that NOEL/

LOELs do not meet the criteria of reporting concentration-response and the only scientifically defensible measure of toxicity is the description of the concentration-response "curve". If toxicity tests are going to be research tools, then the data analysis must accurately describe toxicity. Since the 1970s the "curve" has been demonstrated to provide detailed information including the slope, the ECx values and the error term. Software is available that makes the computation straightforward. Bayesian curve fitting has been developed that allows estimation of a true No Effect Concentration. The Bayesian curve fitting also provides credibility limits for the range of the curve. Since it is clear that the use of the "curve" is the only rational approach we propose 5 recommendations for the SETAC journals. 1. Papers that rely on NOEL/LOELs should not be considered for publication in journals. 2. Curve fitting is the preferred model for concentration/dose response relationships, ideally including confidence intervals and with supporting data. 3. NOEL/LOELs and similar data derivations should not be used as descriptions of concentration/dose-response. 4. Papers that rely on historic NOEL/LOELs should only do so after every attempt has been made to replace these data derivations with concentration/dose-response data. 5. Papers that treat NOEL/LOELs as data for further analysis such as the derivation of SSDs should receive extraordinary scrutiny. Although it is understood that for many tests these are the only results from toxicity tests that remain, the uncertainties in the accurate representation of the concentration-response curves result in unacceptable uncertainty to any inferred information. The broad implications of these recommendations to the journals and the field of environmental toxicology have not escaped us.

**RP102 Characterizing the Influence of Environmental Conditions on the Toxicity of Mine Effluent Constituents to Standard and Non-standard Coldwater Fish** D. Moore, Univ of Guelph, School of Environmental Sciences; D. Poirier, Ontario Ministry of the Environment, Laboratory Services Branch; P. Sibley, Univ of Guelph, School of Environmental Sciences, Dept of Environmental Biology; K. Solomon, Univ of Guelph, Centre for Toxicology, School of Environmental Sciences. A major critique facing the use of standard aquatic toxicology methods is their potential failure to protect against adverse effects seen under non-standard, real-world conditions. This may be, in part, due to their admitted limits found manifested in applied 'uncertainty factors'. However, rather than discard these familiar, proven methods of assessment and their wealth of historical comparative data, current standards should be actively amended and adapted to represent new advances – their uncertainty gaps shrinking as new data becomes available. Our study aimed to characterize the variability of mine-effluent-constituent toxicity across a range of cold-water fish species and environmental conditions to assess whether the current standard test is protective enough to cover this variation. Sixteen contaminants ( $Al^{3+}$ ,  $Cd^{2+}$ ,  $Cr^{3+}$ ,  $Cu^{2+}$ ,  $MoO_4^{2-}$ ,  $Ni^{2+}$ ,  $As^{3+}$ ,  $Se^{2+}$ ,  $NO_2^-$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ,  $NH_4OH$ ,  $KCl$ ,  $MgCl_2$ ,  $CaCl$ ,  $NaCl$ ) were tested using five species of fish native to Northern Canada (rainbow trout, arctic charr, lake trout, lake whitefish, and arctic grayling) across a range of pH (5.5, 6, 6.5, 7, 7.5, and 8), temperature (5, 10, and 15°C), and hardness (30, 60, and 120ppm) conditions and their observed effects modeled as 24, 48, 72, and 96-hour LC50 values. By comparing these 'amended standard' (varied conditions) acute toxicity assay results to those of the standard rainbow trout test (pH 7.5, 120ppm, 15°C), we have characterized the effect of pH, temperature, and water hardness on contaminant toxicity and time-to-toxic effect within and between species while relating these differences to the ecological protection afforded by the current standard risk assessment method. The data suggests that, in some instances, the standard surrogate is not the most sensitive species – but more importantly, that by gathering more data, we can model this sensitivity/toxicity dynamic between species and water conditions so as to create a more accurate and representative standard method.

**RP103 Development and Initial Evaluation of a Reconstituted Water Formulation that Better Represents Natural Waters** S.M. Hoheisel, US Environmental Protection Agency, Student Contract Services; R.J. Erickson, T.L. Highland, J.R. Hockett, US Environmental Protection Agency, Mid-Continent Ecology Division; D. Hoff, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; T.J. Norberg-King, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; T.R. Valenti, US Environmental Protection Agency, National Research Council Grantee; D.R. Mount, US Environmental Protection Agency, ORD. The use of reconstituted waters is deeply entrenched in



many standardized aquatic toxicity testing protocols. The primary appeal of reconstituted waters is inter-laboratory comparability, such that experiments performed in different laboratories can be conducted in (nominally) identical waters. While several formulations of reconstituted waters have been developed over the years, many aquatic toxicity test protocols from the USEPA and ASTM use a formulation that has its roots in a publication by Marking and Dawson (1973). While widely used, the ionic composition of these waters is not typical of North American surface waters; for example, the molar Ca:Mg ratio in EPA/ASTM water is 0.70, but is averages about 2.2 in US waters. More dramatic are the differences in molar Cl:SO<sub>4</sub> ratio, which is 0.0632 in EPA/ASTM water, but is more typically in the range of 1 to 2 in US waters. We have developed a new reconstituted water formulation based on an analysis of natural waters from across the US. Hardness was chosen as the master variable from which the remaining composition is calculated. The subsequent relationships that define the water composition are alkalinity v. hardness, Ca:Mg ratio, Cl:SO<sub>4</sub> ratio v. hardness, and K as a proportion of Na + K. Exactly mimicking natural waters is tedious in the laboratory, because natural waters gain alkalinity from calcium and magnesium carbonates, salts which are difficult to dissolve; this formulation uses only readily soluble salts, though this logistical convenience sacrifices some fidelity to the central tendency of natural waters. Recipes for water of any hardness (recommended bounds of 10 to 400 mg/L as calcium carbonate) can be calculated in a simple spreadsheet. Initial evaluation of these waters in chronic tests with *Ceriodaphnia dubia* and *Daphnia magna*, and short-term chronic tests with fathead minnows (*Pimephales promelas*), have shown performance in our proposed reconstituted water to compare favorably with Marking and Dawson-based waters or natural Lake Superior water. Additional testing with *Chironomus dilutus* and *Hyalella azteca* is planned. Using reconstituted waters that are more representative of natural waters should improve assessment of chemicals whose toxicity is influenced by the ionic composition of the water (e.g., many cationic metals), and may also improve performance of some organisms found to be sensitive to the ionic composition of water (e.g., *Hyalella*).

**RP104 Evaluation of the Influence of Bromide or Iodide on the Performance of the Amphipod *Hyalella azteca* in Reconstituted Waters** C.D. Ivey, US Geological Survey, Columbia Environmental Research Center, USGS – CERC, Dept of Toxicology; C.G. Ingersoll, USGS, Columbia Environmental Research Center; N.E. Kemble, US Geological Survey, Columbia Environmental Research Center, USGS – Biological Resources Division, Columbia Environmental Research Center; J.L. Kunz, USGS; D.R. Mount, US Environmental Protection Agency, ORD; R.J. Hockett, US Environmental Protection Agency. Survival, growth, or reproduction of the amphipod *Hyalella azteca* (HA) is reported to be poor when some reconstituted waters have been used to conduct chronic (>14-d) water-only or sediment toxicity tests; including ASTM reconstituted hard water (with no addition of Bromide). USEPA held a workshop in Chicago in March 2010 with participants experienced in culture or testing of HA in water or in sediment. Participants at the workshop reported good performance of HA cultured or tested in Borgmann (1996) reconstituted water (with the addition of Bromide). In contrast, limited success was reported for laboratories conducting long-term water-only exposures of HA in Smith et al. (1997) reconstituted water (with no addition of Bromide). The suggestion was made at the workshop to evaluate Iodide based on the conclusion that the protective mechanism of Bromide and Iodide may be similar in improving performance of HA in reconstituted waters. The influence of Bromide (Br as NaBr) or Iodide (I as NaI) on the response of the HA under water-only 42-d exposures was evaluated in Borgmann or Smith et al. reconstituted waters along with well water (300 hard) and well water diluted with deionized water to 100 mg/L hardness as CaCO<sub>3</sub> (100 hard). Endpoints included 42-d survival, growth, biomass, and reproduction. Bromide was tested at three concentrations (0, 0.08 and 0.8 mg Br/L) in Borgmann, Smith et al., and 100 hard waters (0.8 mg Br/L is the concentration recommended for Borgmann water). Iodide was tested at three concentrations (0, 0.005 and 0.05 µg I/L) in Borgmann and Smith et al. waters. Good performance of HA was observed in the 300 hard and 100 hard waters, and addition of Br did not improve performance of HA in 100 hard water. Addition of Br or I in Borgmann water increased survival, growth, biomass, and number of young, with Br having a stronger influence compared to I. Good performance of HA was observed in Smith et al. water without the addition of Br or I which may have been due to an increase in the amount in YCT fed (up to 3 ml/day). In general the addition of Br (at 0.08 or 0.8 mg Br/L) had a stronger

influence on the performance of HA compared to addition of I. Additional testing with Br is planned to determine if environmentally lower and more relevant concentrations of Br might improve performance of HA in chronic water or sediment exposures with various reconstituted waters.

**RP105 Freshwater Mussel Growth and Survival in Sediments Influenced by Agricultural and Industrial Land Use** P.D. Hazelton, Univ of Georgia, Warnell School of Forestry & Natural Resources, Univ of Georgia, graduate research assistant; R. Bringolf, Univ of Georgia, Interdisciplinary Toxicology; M.L. Urich, USGS-Patuxent Wildlife Research Ctr., Warnell School of Forestry and Natural Resource; P.J. Lasier, USGS-Patuxent Wildlife Research Ctr., Univ of Georgia. Freshwater mussels (order Unioniformes) are among the most critically imperiled taxa worldwide. In North America, greater than 70% of the native mussel species are listed as Endangered, Threatened, or species of special concern, and environmental pollution is often cited as a major contributor to this loss of diversity. To date, standardized methods and most toxicity research on freshwater mussels have focused on the effects of water borne contaminants on the survival of larval and juvenile stages within the mussel lifecycle. However, juvenile mussels acquire the majority of their nutrition through pedal feeding in the sediment and exposure to contaminants through sediments and pore water is likely a more realistic route of toxicity than through water-only exposures. We used existing methodologies for 28-day sediment-toxicity assessments to investigate the effects of sediments from a river impacted by agricultural and industrial land use on the growth and survival of juvenile (approx. 4 month old) mussels (fatmucket, *Lampsilis siliquoidea*). Sediments were collected from sites along the Conasauga River (northwest Georgia, US) that receive agricultural runoff and perfluorinated compound (PFC) pollution. A reference sediment was collected from outside of the watershed. We normalized effects of confounding sediment characteristics (i.e., total organic carbon, sediment size) as statistical covariates, and therefore isolated the effects of the contaminated sediment. Growth and survival differed among sites, leading to identification of river reaches that are most impacted by the various land uses. Results from our assay are particularly useful for ecological risk assessment since they incorporate exposures to environmentally realistic contaminant concentrations but minimize the confounding variability and logistic complexity of in situ studies.

**RP106 It is Time for a Paradigm Shift in the Analysis of Toxicity Data for Regulatory Applications** J. Diamond, Tetra Tech, Inc.; D. Denton, USEPA Region 9, c/o SWRCB. The whole effluent toxicity (WET) program in the US currently requires multi-concentration testing of effluents. While multi-concentration testing of chemicals is desirable for regulatory and scientific reasons, we believe this requirement is not as efficient for evaluating effluent compliance in a WET program. The key regulatory question of concern is whether an effluent is toxic or not, which is best answered statistically using a hypothesis approach, not a point estimate approach. However, the traditional hypothesis approach currently recommended does not reward high within-test precision. This paper describes the need for a paradigm shift in the analysis of WET compliance data consisting of three parts: (1) restate the null hypothesis so that test power is now associated with demonstrating that the effluent is not toxic; (2) use EPA's Test of Significant Toxicity (based on non-inferiority or bioequivalence approach) to define what is considered unacceptable toxicity as well as acceptable effects and to identify each condition with a high probability; and (3) evaluate only the test control and the critical concentration of concern (e.g., instream waste concentration). We demonstrate that together, instituting these three changes would provide: positive incentives for permittees to produce high quality WET data; a transparent analysis approach in which the permittee could have greater control over regulatory decisions based on WET test results; and potentially a less expensive testing program because fewer effluent concentrations need to be examined within a test. As a result, WET test frequency or increased within-test replication could be increased for the same cost as current testing programs while providing better characterization of effluent quality.

**RP107 The Stream Pollution Trends (SPoT) Program: A Framework for Monitoring Long-term Water Quality Trends in California's Watersheds** K. Siegler, Univ of California – Davis, Environmental Toxicology; J. Hunt, Univ of California – Davis, Dept of Environmental Toxicology; B. Phillips, Univ of California – Davis, Environmental Toxicology; B. Anderson, Univ of California – Davis, Dept of Environmental Toxicology; J. Voorhees,

University of California – Davis, MPSTL – Environmental Toxicology; R. Tjeerdema, Univ of California – Davis, Environmental Toxicology. The Stream Pollution Trends (SPoT) program is a California statewide monitoring program designed to determine long-term trends in stream contaminant concentrations and effects, relate water quality indicators to land-use characteristics, and to establish a network of sites throughout the state to use in collaboration with local, regional, and federal monitoring programs. Sites at the base of 96 watersheds have been monitored for sediment toxicity and a suite of pesticides, trace metals, and industrial compounds since 2008. In 2008 23% of the sediments were significantly toxic to *Hyalella azteca* in 10-day tests when tested at 23 degrees Celsius. This trend continued in 2009 (39%) and 2010 (28%). Percent land use was calculated throughout the watersheds, and urban land uses had the most significant correlations with contaminant concentrations. Sediment toxicity was also significantly correlated with mixtures of pyrethroid pesticides. In 2010 a subset of 24 sites was also assessed using amphipod tests conducted at 15 degrees. Twice as many samples were toxic when tested at 15 degrees (42%), suggesting pyrethroid pesticides contributed to the observed toxicity. Data from this program are intended to detect changes in contamination and toxicity as management initiatives are implemented to reduce pesticide loading in California watersheds.

**RP108 Use of Field Collected Aquatic Macroinvertebrates to Determine Acute Zinc Toxicity** S.M. Pargee, GEI Consultants, Inc; S.P. Canton, GEI Consultants, Inc., Ecological Division. Toxicity of zinc to typically sensitive aquatic macroinvertebrates (mayflies, stoneflies, and caddisflies or “EPT taxa”) in Colorado streams is relatively unknown and of particular interest when considering applicable zinc standards for coldwater trout streams. The lack of laboratory-derived zinc toxicity data using standard test methods for aquatic macroinvertebrates is likely due to unavailability of cultured test organisms and uncertainty associated with field collected organisms. We successfully collected, transported, and acclimated a mayfly (*Drunella grandis*), a stonefly (*Isoptera* sp.), and a case dwelling caddisfly (*Lepidostoma* sp.) from the South Fork Cache la Poudre River, Colorado, a stream unaffected by heavy metals from mining activities. We were unable to run tests with two other collected aquatic insects, the mayfly *Baetis tricaudatus* and the free-living caddisfly *Dolophilodes aequalis*, either as a result of sensitivity to handling stress from collection, transport, and test set up (*Baetis*), or inability to acclimate to laboratory test conditions (*Dolophilodes*). Static-renewal 96-hr acute toxicity tests were conducted using standard test methods in soft, hard, and very hard reconstituted lab water. Differences in toxicity and acclimation to laboratory conditions were observed within and between Orders. *Drunella grandis* performed very well both in the field collection phase and under laboratory conditions (LC50 >6.29 mg/L in hard water and >3.05 mg/L in soft water). Similarly, *Lepidostoma* sp. performed well (LC50 >38.8 mg/L in hard water and >81.7 mg/L in very hard water). No acute toxicity was observed for zinc concentrations up to 27 mg/L for *Isoptera* sp. Overall, toxicity values for these field collected EPT taxa were much greater than predictions based on comparisons to other sensitive macroinvertebrates or zinc tolerance values reported from field observations of aquatic insects near inputs of heavy metals (e.g., mining areas). This study demonstrated that with proper handling, and species specific considerations, use of field collected native species may be a valuable tool to help assess toxicity in aquatic environments.

**RP109 Use of Water-only Chronic Toxicity Test Methods to Compare Life History Characteristics of Four Genetically Distinct Populations of *Hyalella azteca*** K. Major, Univ of Illinois at Urbana-Champaign, Illinois Natural History Survey, Illinois Natural History Survey; D.J. Soucek, R. Giordano, Univ of Illinois at Urbana-Champaign, Illinois Natural History Survey. The epibenthic amphipod *Hyalella azteca* has long been used as a model organism for toxicity bioassays because of its importance to food webs, its adaptability to culture, and its wide distribution across North America. However, current morphological identification of this amphipod is proving to be insufficiently descriptive. Recent studies using allozymes and/or mitochondrial cytochrome *c* oxidase (COI) gene sequences have provided evidence for the existence of numerous undescribed species within wild North American populations of *H. azteca*. Furthermore, the extent to which *H. azteca* laboratory populations are accurate representatives of wild populations has not been established. Because life history characteristics are the basis for chronic toxicity test endpoints, identifying differences in these characteristics among different populations is critical. Therefore, our

goal was to collect samples from various *H. azteca* populations, identify the populations genetically by sequencing the entire COI gene, and investigate whether genetically distinct populations differed in terms of life history attributes. First, we determined complete COI sequences for wild populations of *H. azteca* from sites in the US and Canada, as well as for several laboratory populations from commercial sources, US and Canadian regulatory agency laboratories, and academic research groups. Phylogenetic analysis with maximum parsimony yielded five distinct clades. Each of the five clades exhibited low within-clade percent divergence (< 3%), indicating that members of the same clade were conspecific. However, high across-clade percent divergences (17-20%) indicated that all clades exhibited species-level divergence from one another based on average COI divergence rates among other crustaceans. Next, we sought to compare life history characteristics of the different clades. Representative populations from four of the five clades were acclimated to laboratory conditions for at least three generations to eliminate potential differences due to environmental and/or maternal effects. 42-day chronic water-only toxicity test methods were used (with populations acting as treatments) to show that in addition to genetic differences among these clades, life history characteristics, namely body mass and male-to-female body mass ratio, differed significantly.

**RP110 What Food and Feeding Rates Are Optimum for the *Chironomus dilutus* Sediment Toxicity Test Method?** T.J. Norberg-King, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division; T.L. Highland, J.R. Hockett, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division; D.R. Mount, US Environmental Protection Agency, ORD; T.W. Valenti, National Research Council; US Environmental Protection Agency, National Research Council. Laboratory tests with benthic macroinvertebrates are commonly used to assess the toxicity of both contaminated sediments and individual chemicals. Among the standard procedures for benthic macroinvertebrates are 10-d, 20-d, and life cycle exposures using the midge, *Chironomus dilutus*. This presentation describes a series of studies conducted to evaluate and optimize feeding regimes for midge toxicity testing. Current ASTM and USEPA test guidelines specify feeding a fine suspension of flake fish food (i.e., blended in water) at a rate of 6 mg dwt/day to each replicate chamber of 10 organisms. In 10-d exposures in which the initial stocking density varied from 6 to 12 organisms per replicate, the final weight of larvae increased markedly as stocking density decreased, suggesting that food quantity or accessibility does limit midge growth under standard test conditions. Additional experiments evaluating higher feeding rates confirmed higher midge growth is obtained if daily rations were increased. However, higher rations of the standard food preparation also result in the depression of dissolved oxygen, which in turn can limit midge survival and growth. Experiments examining several other diets and preparations did not identify an alternative food source that supports both strong larval growth and not create problematic oxygen demand. However, when presenting flake fish food as a suspension of fine flakes (passing through a No. 50 sieve) rather than the blended preparation we observed improved growth. The fine flake food may have allowed food particles to more readily settle to the sediment surface as these treatments yielded high growth rates with lower feeding rates. A series of 10-d toxicity tests were conducted and subsamples for growth measurements were taken at 4, 7, and 10 d of the testing exposure in an effort to optimize a progressively increasing ration design. We discuss the implications of these findings for midge toxicity tests and provide recommendations on improved feeding regimes. Note: This abstract does not necessarily reflect USEPA policy.

**RP111 Effective Risk Communication to an Ever Increasing Cynical Public** A.E. Goldberg Day, ARCADIS, RAES. Risk communications became prominent in the mid-1980s, when regulatory agencies realized that the risk management policies proposed by environmental specialists often were complex and were not necessarily acceptable to the wider public. Since that time, risk communication has evolved from human health risk assessment methodology from research on how individuals perceive risk. It is defined as an interactive process of exchange of information and opinion among individuals, groups, and institutions. It often involves multiple messages about the nature of risk or expressing concerns, opinions, or reactions to risk messages, or to legal and institutional arrangements for management. During the presentation, the USEPA's key rules for effective communication will be presented. The goal of the discussion is for the attendees to gain tools to aid them in their own risk communication endeavors. KEY RULES FOR

**EFFECTIVE COMMUNICATION** (from the USEPA) • Accept and involve the public/other consumers as legitimate partners. • Plan carefully and evaluate your efforts with a focus on your strengths, weaknesses, opportunities, and threats. • Listen to the public's specific concerns. • Be honest, frank, and open. • Coordinate and collaborate with other credible sources. • Meet the needs of the media. • Speak clearly and with compassion.

**RP113 Life Cycle for Microalgae-based Biofuels: Reducing Footprint and Production Costs by Using Waste from Sugar/Ethanol Plants as Raw Material** J.A. Neto, H.L. Maranduba, Santa Cruz Univ, Dept of Agricultural and Environmental Sciences; I.A. Nascimento, Federal Univ of Bahia, Dept of Biology. In the actual world energy scenario, ethanol and biodiesel are pointed out as partial substitutes for petroleum products to attend the transportation sector. Regarding ethanol, Brazil ranks second worldwide with a production of 31 billion L, while for biodiesel the installed production capacity is 5.5 million L/year, able to replace diesel by 5%, according to National Energy Policies. This replacement is attractive due to the availability of agricultural land and the wider variety of oil-seed species. However, the expansion of crops leading to land use changes, may release the CO<sub>2</sub> stored in soil and nitrogen gases, intensifying the GHGs effects, thus negating the benefits of biofuels to curb emissions. Microalgae emerge as a viable solution as feedstock for biofuels. In relation to land-crops, microalgae have higher productivity and higher capacity of CO<sub>2</sub> sequestration. However, the proliferation of microalgae cultivation systems depend on their footprints and production costs reduction. Based on life cycle analysis and converting all GHGs and energy usage to tone of carbon dioxide equivalent/year, three alternatives to the two isolated processes of producing ethanol from sugar-cane and biodiesel from microalgae cultivated in open ponds have been compared to different associations of both processes: (i) biodiesel production based on microalgae biomass output, by using CO<sub>2</sub> from ethanol production and biodigested vinasse as source of nutrients; (ii)-the same conditions as alternative (i), plus the harvest water recycling; (iii)-the same conditions as (i) and (ii), plus the production of methane from the residual biomass and carbon-free electricity. The results highlight the benefits of all the three alternatives involving the co-production of biofuels from microalgae cultivation process inserted into the sugar/ethanol plants and indicate the approach (iii) as the most viable to reduce production costs and to decrease biodiesel and ethanol footprints.

**RP114 Study on the Greenhouse Effect Assessment of Land Use Based on LCA** S. Chen, X. Yang, Beijing Univ of Technology. The greenhouse gas emissions from land use every year are 11 times the emissions from fossil fuel. The greenhouse effect caused by the process of land use on global climate change is crucial. In this study, based on the impact assessment method of land use in life cycle assessment and models of IPCC, the calculation models of GHG emissions of two types land use including land occupation and land transformation were established. According to the proposed models and land use situation in China the classifications of land are natural forests, man-made forests, grassland, paddy fields and dry farmland. Based on the study of GHG emission regarding of different types land in various climatic zone, we proposed the greenhouse effect factors of different types land in various climatic zone in China. We also summed up the length of one type land being transformed to another in China. In addition, with the land occupation of buildings and the land transformation of forests being taken as case studies, the greenhouse effects of land use were estimated using the proposed models in China. The research is sponsored by the Twelfth Five-year National Science & Technology Support Program (NO. 2011BAC04B03 & NO. 2011BAC04B04)

**RP115 Modeling Biodiversity Impact into Environmental Life Cycle Assessment** B. Neupane, Univ of Maine, graduate research assistant; A. Halog, Univ of Maine, Research Group of Industrial Ecology, LCA and System Sustainability (IELCASS), Univ of Maine, Assistant Professor of Industrial Ecology and LCA. A clean and sustainable source of energy is important for economic development of a nation. One of the tools to assess the environmental sustainability of energy systems is life cycle assessment (LCA). The major life cycle impact categories considered in current LCA practice include climate change potential, acidification potential, eutrophication potential, photochemical oxidants, fossil fuel depletion, fresh water toxicity, etc. This, however, does not account for biodiversity impacts and land use implications. The study attempts to develop a framework to incorporate biodiversity impact category into conventional LCA method. This

framework is applied in the context of woodchips production used in biorefinery for ethanol production. In the proposed framework, the Landscape Management System (LMS) tool is used to model forest management activities for a set of harvesting systems under consideration. Forest stands level information, such as habitat type and stand-development stages, are used to prepare a habitat-species relationship matrix. This matrix information was incorporated into LCA inventory modeling to prepare a set of characterization factors. Habitat type classifications and corresponding area calculations are spatially represented using Geographical Information Systems (GIS). Different biodiversity impact indicators (e.g., species richness and habitat naturalness, etc.) are used to model the biodiversity impact. The results show that biodiversity impact increases as the ethanol blending proportion to gasoline is increased. The potential biodiversity impact is found to be higher in the first decade of the considered time frame, and it shows a flattening trend towards the next decades. From our analysis, it can be concluded that when a whole rotation length of forest is considered, the potential biodiversity impact is relatively unchanged after the first few decades.

**RP116 Spatially Differentiated Worldwide Midpoint and Endpoint Characterisation Factors for Acidification** P. Roy, CIRAIG, CIRAIG; L. Azevedo, M. Huijbregts, Radboud Univ; L. Deschenes, M. Margni, M. Margni, CIRAIG. With the globalization of markets, assuming that the entire life cycle emissions of a product system occur within a specific geographical area (e.g., Europe or Canada) is no longer a suitable assumption for regional impact categories, since the impact of a same elementary flow varies from an emission location to another. Furthermore, current LCIA approaches do not allow to consistently assess and compare emissions occurring from different continents as the characterization factors (CF) are obtained from different characterization models. Thus, a model covering a worldwide scale, but also able to account for local conditions, needs to be developed to provide consistent regionalised CFs worldwide. This work presents a new global characterization model able to consistently evaluate acidifying emissions at both the local and global scale for the acidification impact category. The emission location based midpoint and endpoint CFs for emissions of NO<sub>x</sub>, SO<sub>2</sub>/SO<sub>4</sub> and NH<sub>3</sub> were obtained on a 2x2.5° (latitude x longitude) spatial grid resolution. The CFs from this resolution were then aggregated to provide other CFs at coarser resolutions: worldwide, continental and country level (states and provinces resolution were also provided for the US and Canada). Midpoint CFs were obtained from the multiplication of an atmospheric fate factor (FF) and a receiving environment sensitivity factor (SF). FFs [keq deposited/ha/yr, kg N or S emitted/yr] derivation was based on the global atmospheric model GEOS Chem. SFs [mol/L ha, keq deposited /ha/yr or ha, keq deposited/ha/yr] were evaluated through the variation, caused by a marginal change in emission/deposition, of, respectively, two different soil chemical indicators: H<sup>+</sup> concentration (linked to pH) and the base cations (Ca<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>) on aluminum (Al) ratio. Variations in soil chemical indicators were evaluated using the PROFILE steady-state model and a developed global dataset. Endpoint CFs were obtained from the multiplication of the previously mentioned FFs and SFs with an effect factor (EF). The biome specific EF [-, mol/L] curves links change in the potential occurrence of species (PNOF) due to a change in soil's pH. The results showed that both midpoint and endpoint CFs were highest at emission location where soils have limited buffer capacity (e.g., boreal forest).

**RP117 Use of Lifecycle Tools to Optimize Sustainable Energy Campuses** J. Fava, Five Winds International; E. Beaver, Carbolytic Materials Company; S. Baer, PE International, 20 Paoli Pike. This paper describes the use of GaBi (TM) Life Cycle software to optimize the design of a Sustainable Energy Campus based upon feed of scrap automobile and truck tires. Further, once the Sustainable Energy campus is defined, the software and related tools are used to compute the benefits of reduced lifecycle consumption of fossil fuels, emission of greenhouse gases and other wastes and the overall energy savings. The SEC combines multiple technologies from different industries to create a truly sustainable "waste-to-profit" campus where the waste from one technology becomes the input to another technology that utilizes that waste to create highly profitable fuel products. GHG are reduced, materials recovered, energy optimized and virtually no emissions. Further, the reality is highly profitable. Thus, waste-to-profit. Technologies from the rubber, chemical and petroleum industries are at play in the Sustainable Energy Campus. A Carbon Black recovery production line takes scrap tires and uses a vacuum pyrolysis process to convert those tires into



a product called Carbon Black Alternative and a stream of pyrolysis oil. Carbon Black is present in 75-85% of all new tires produced worldwide. A cracking and reaction technology combined with a sophisticated separation process converts the pyrolysis oil into raw materials for gasoline, kerosene, diesel, naphtha, jet A fuel base and liquified low molecular weight hydrocarbons (propane, butane and pentane). While each step can be profitable independently, combining them in a single site with cogeneration allows for energy and materials optimization as well as maximizing revenue. Further, minor components are captured and isolated which have extremely high value. The key materials are cyclic terpenes and high been recovered at high yields and high purities. A single scrap tire weighs approximately 21 pounds and one tire is produced per year per person in industrialized countries, the SEC provides a means of producing massive amounts of valuable products which otherwise would be produced from incremental fossil fuels and raw materials at significant cost to society and the environment. A full scale tire pyrolysis unit utilizing this technology has been in operation for more than a year producing more than ten million pounds of carbon black along with the co-products. The data related to mass of products, energy savings and net environmental impact will be presented and discussed.

**RP118 Normalization and Weighting Using Multi-criteria Decision Aids Under High Uncertainty** T. Seager, Arizona State Univ, School of Sustainable Engineering & Built Environment; V. Prado, Arizona State Univ, School of Sustainable Engineering & the Built Environment; S. Tylock, Sustainable Intelligence, LLC. Existing ISO recommendations call for *external* normalization of characterized life cycle inventories relative to nation- or multi-nation accounts, such that all mid-point categories are expressed as a dimensionless fraction. This approach is problematic in several respects, especially in cases where the primary purpose of the life cycle assessment is for comparing emerging or prospective technological alternatives. For example, previous research has demonstrated that the ISO approach can mask aspects of a life-cycle decision that may be critically important. Moreover, ISO provides little guidance on handling uncertainty – either in reference data or in weight sets. This presentation demonstrates a novel approach to normalization and weighting in impact assessment that is especially applicable to conditions of novel technology comparison that adapts established methods of multi-criteria decision analysis developed to support environmental management. Most notably, the new method uses *internal* normalization, thereby obviating the need for a reference dataset, and is designed specifically to allow stakeholders to explore the sensitivity of preferred rank orderings to uncertainties of several types, including weight sets. The results can potentially facilitate deliberative dialogue among stakeholders with competing value systems, or serve to prioritize additional research. We propose that the new method be adopted at the earliest stages of product or policy development, where both the uncertainties and the stakes may be highest.

**RP119 Bridging Generic Inventory with Spatialized Characterization Factors: First Results** G. Bourgault, P. Lesage, R. Samson, CIRAI. Spatialization of impact assessment methods results, for each elementary flow, in a set of characterization factors (CFs) whose choice depends on the longitude/latitude coordinate of the emission. Regardless of the impact category, recent findings revealed that variability of CFs over space covers several orders of magnitude when using a subwatershed or very fine air grid delineation. Inventory, on the other hand, typically provides spatial information on emissions at a much coarser resolution, for example at the country level. This situation is likely to stay unchanged in the near future. In the absence of the necessary spatial information in the inventory, a choice has to be made in the set of CFs for each emission. Since they cover such a wide range of values, the outcome of the LCA is likely to be sensitive to the choice, and the criteria for choosing particular CFs should be transparent and rigorous. In the present project, TRI (USA) and NPRI (Canada) are used to localize each emission site for each elementary flow. This localization allows for a) proper weighting of the spatialized CFs in the calculation of a site-generic CFs based on the spatialized CFs, and b) a quantification of the uncertainty created by the lack of spatial information in generic databases. Preliminary results show a very unequal distribution of emissions throughout North America, underlining the necessity of taking into account this information in the calculation of a site-generic CFs.

**RP120 Creating a US National Standard for Life-Cycle Environmental Performance Assessment** B.H. Karsell, Independent Environmental Consultant. A committee is developing a US national standard for type III

life-cycle impact profile declarations for products, services and systems sufficient to support public declarations and claims. The committee is following the ANSI standard development process, and is sponsored by the Leonardo Academy, the only ANSI-accredited standard development organization to require participation by environmental interests. The standard will include the following attributes: Compliant with ISO 14044 Use best available science Quantify all relevant environmental impacts Include no value judgments Allow no aggregation of impacts among impact categories Produce consistent and auditable results To date the draft standard includes 25 different categories of environmental impacts. The committee is actively seeking engagement with and vetting of the standard practice by all interested stakeholders. We expect to produce a final standard in 2012.

**RP121 Field and Laboratory Boron Toxicity Results and New Understanding of Boron Bioavailability, Fate and Transport: Implications for Guideline Development** G.J. Huber, A.L. Knafla, Equilibrium Environmental Inc.; D. Lintott, Exova. Boron is a metalloid micro-nutrient for plants which can also be phyto-toxic at elevated concentrations. It is a common co-contaminant found with salts such as sodium chloride, and may be introduced into soil and groundwater from produced water from oil and gas operations. It is also used in agriculture as a fertilizer, and in industrial processes such as fiberglass manufacturing. Boron can also be found in elevated concentrations in groundwater in arid regions such as California, where irrigation with boron-laden groundwater can cause significant toxic effects on plants. Soil quality guidelines for boron are currently based on older plant toxicity data, with limited integration between toxicity data from irrigation water and measured concentrations in soil. Consequently, research has been performed over the last several years in Canada with the aim of developing updated, risk-based soil quality guidelines for boron. These would integrate updated plant toxicity and bioavailability results along with enhanced groundwater fate and transport modeling. The updated plant toxicity testing was based on a combination of field and laboratory tests, with laboratory toxicity testing protocol from Environment Canada providing standardized methodologies for evaluating adverse effects on plant growth. These laboratory toxicity tests suggest that toxic thresholds may be significantly higher than the Canadian soil quality guidelines of 2 mg/kg "hot-water soluble" (HWS) boron. Recent field studies performed with a variety of agricultural species also suggest that toxic thresholds are above existing soil guidelines. Traditional soil tests such as the HWS method were compared to potential alternative test methods to evaluate their relevance to the bioavailability of boron to plants and other receptors along with fate and transport properties. The HWS boron methodology was found to be unsuitable for measuring the bioavailable amount of boron in soil to terrestrial rodent wildlife species, livestock, humans, and plants, with alternative test methods showing improved relevance. The HWS methodology was also found to not provide a good representation of the amount of mobile boron in groundwater. Enhanced transport modeling based on alternative soil tests to other groundwater receptors such as aquifers and surface aquatic systems will allow integration of drinking water guidelines, irrigation guidelines, and aquatic life guidelines into updated soil guidelines in the future.

**RP122 Variation in Metal Bioaccessibility in the Canadian Maritimes** M. Dodd, Royal Roads Univ, School of Environment & Sustainability, Royal Roads Univ, School of Environment & Sustainability; A. Rencz, Natural Resources Canada – Geological Survey of Canada, Geological Survey of Canada; P. Friske, Natural Resources Canada – Geological Survey of Canada; W. Beirsto, Golder Associates. The North American Geochemical Landscapes Project was initiated to provide a soil geochemical database for a broad-based group of users in the field of environmental and human health. The survey is based on low-density sampling (within a 40 km by 40 km grid) in Canada, the United States and Mexico yielding 13,212 sites in total. This Tri-national survey will ultimately produce a database of the regional natural-occurring differences in concentrations and physicochemical characteristics which can be used to assess background conditions and identify anthropogenic impacts. The Canadian part of the project was initiated by Natural Resources Canada – Geological Survey of Canada (GSC) in partnership with other provincial and federal agencies including Health Canada. Sampling has been completed in the Canadian Maritimes comprising Nova Scotia, New Brunswick, Prince Edward Island and Newfoundland. A sub-set of the samples collected from the surface (0-5 cm), B and C horizons were analyzed for metal bioaccessibility using a simplified physiologically based extraction test as a surrogate for bioavailability. The data indicated variation

in metal bioaccessibility among the provinces. Differences in metal bioaccessibility for the three soil horizons were also noted. There were also some statistically significant relationships between metal bioaccessibility and geochemical data including total metals, soil pH, total organic carbon and loss on ignition. It is anticipated that this data will form the basis for the continuing studies aimed at completing a national picture of metal bioaccessibility in background soil samples and its relevance to human and ecological health risk assessment in Canada.

**RP123 Geochemical and Biological Factors Allow Better Understanding of Sedimentary As, Cd and Cr Uptake in a Deposit-feeding Polychaete *Nereis succinea*** Z. Baumann, N. Fisher, Stony Brook Univ, School of Marine and Atmospheric Sciences. Contaminant metals are not irreversibly bound to sediment, hence they can pose risks to resident biota and, following trophic transfer, they can also pose human health risks from seafood consumption. It is therefore important to understand the extent, pathways and mechanisms of toxic metal bioaccumulation in contaminated coastal waters. To better quantify metal bioaccumulation in a benthic invertebrate, the assimilation efficiency (AE) of ingested As(V), Cd, and Cr(III) were assessed in the deposit-feeding polychaete *Nereis succinea* feeding on estuarine sediments. Uptake of metals from pore water and efflux rate constants following dietary or aqueous metal exposures were also determined. To measure metal concentrations, water, sediments and polychaetes were collected from three different estuarine sites – two in Chesapeake Bay and one in San Francisco Bay. Patterns of geochemical partitioning of As, Cd and Cr were also determined in the laboratory and were linked to observed bioaccumulation of these metals in the field-sampled polychaetes with good correlations ( $r^2 = 0.84 - 0.87$ ). To incorporate the geochemical link into bioaccumulation modeling we modified a commonly used bioaccumulation model by incorporating the geochemical fractionation of the metals in the sediments. The model indicated that >97% of metals are acquired by worms via ingestion. To further explore the ingestion pathway, we investigated possible mechanisms of As assimilation in *N. succinea*. The release of As from sediment particles into gut fluid extracted from *N. succinea* was related to As AEs, however As released from inorganic particles (e.g., goethite) was largely unassimilated, whereas As bound to organic compounds and released from algal cells was assimilated very efficiently. Thus, the likelihood of As assimilation is higher when it is bound to an organic compound that is of nutritional value to the polychaete.

**RP124 Impact of the Earthworm *Lumbricus terrestris* on the Mobility and Speciation of Arsenic** T. Sizmur, Univ of Reading, Soil Research Centre, Univ of Reading, Soil Science Dept; M. Watts, B. Palumbo-Roe, British Geological Survey; J. Charnock, Univ of Manchester; M. Hodson, Univ of Reading. Human activities have resulted in an increase in the concentrations of As in both urban and rural soils. In order to properly assess the risks that As poses to the environment a greater understanding on how soil biota influence the fate of As in soil is required. The bioavailability of As in soil is greatly dependent on its mobility and speciation but little is known about the effect of passage through the earthworm gut on the mobility and speciation of As. Earthworms burrow in the soil and create casts that are chemically different to the surrounding soil. These casts are often produced on the surface of the soil and there is therefore potential for As to be leached out of the casts into water courses at a rate greater than that predicted by models used in risk assessment. We carried out experiments to determine the impact of passage through the gut of a UK native anecic earthworm; *Lumbricus terrestris* L. on the mobility and speciation of As in soil (1130 mg kg<sup>-1</sup> As) collected at Devon Great Consols, an abandoned Cu and As mine situated on the bank of the river Tamar, UK. *L. terrestris* were cultivated in the As-contaminated soil and the casts collected. Water soluble As increased from 1.6 mg kg<sup>-1</sup> in the bulk soil to 18 mg kg<sup>-1</sup> in the casts. Casts were then aged and this effect was still present 56 days after soil excretion. HPLC-ICP-MS analysis coupled with synchrotron based techniques (EXAFS/XANES) indicated that no reduction of As(V) to As(III) occurred, despite passage of the As through the anoxic rear-gut of the earthworm. Observed increases in As mobility in casts were due to release of As(V) from soil particles during the digestion process as a result of increases in pH and increased concentrations of dissolved organic carbon. The mobilisation of As (and other elements) from contaminated soils in the environment by cast production may allow for accelerated leaching or uptake into biota which is under-estimated in current risk assessments and models based on analysis using soils in

the absence of soil biota. This research therefore has implications for the risk assessment of As contaminated sites and for As biogeochemical cycling.

**RP125 The Effect of Road Salt on the Metal Binding Ability of Adsorptive Media** A. Whiting, Towson Univ; R. Casey, Towson Univ, Urban Environmental Biogeochemistry Laboratory, Towson Univ, Dept of Chemistry; S.M. Lev, D. Ownby, Towson Univ, Urban Environmental Biogeochemistry Laboratory. Many studies have verified the connection between increasing urbanization and declining water quality in tributaries of the Chesapeake Bay. Urban stormwater runoff is currently one of the most significant causes of aquatic habitat degradation. To mitigate the effects of stormwater, researchers have studied the effectiveness of different media as stormwater filters. However, the effect of road salt on the effectiveness of those media is often overlooked. This project evaluated possible media solutions to road salt-impacted stormwater runoff. Stormwater was collected from the scuppers of two highway bridges and analyzed for dissolved ions, metals, and total suspended solids. Highest metal and salt concentrations collected were 95 µg/L Zn, 55 µg/L Cu, 48 µg/L Cr, 39 µg/L Pb, 4 µg/L Ni, 1713 mg/L Na and 2609 mg/L Cl. The second part of the project evaluated filter media provided by Contech Construction Inc. and Imbrium Systems, Inc. to remove stormwater contaminants from runoff before they reach the aquatic environment. Batch tests were conducted to quantify media effectiveness. Treatments for the batch tests included three salt concentrations (none, 2.5 g/L, 5 g/L NaCl) mixed with a standard combination of metals as their nitrate salts (500 µg/L Zn, 100 µg/L Cu, 50 µg/L Pb, 50 µg/L Cr, and 50 µg/L Ni). Metals and salt concentrations used in the treatments were reflective of values found in the highway bridge runoff sampled. Batch tests included two blank tests (deionized water and artificial stormwater with no metals or salt added). Deionized water exchanges revealed that all Imbrium media released Na<sup>+</sup> (3.5 – 1181 mg/L), and all but one released SO<sub>4</sub><sup>2-</sup> (3 – 2929 mg/L). A zeolite tested released a small amount of Zn (10 µg/L) while a compost based media released 80 mg/L K<sup>+</sup> and 10 mg/L Cl<sup>-</sup> on average. After completion of batch tests, column studies will be conducted on the most effective media.

**RP126 Weathering Transformations of Sorption Properties of Biosolids for Metals and Their Possible Environmental Implications** I. Twardowska, E. Miszczak, S. Stefaniak, Polish Academy of Sciences, Institute of Environmental Engineering. Stabilized sewage sludge has a long tradition of use in agriculture as biosolids (BS) in North America. In the European Union, the environmentally safe way of its application in agriculture is encouraged by the Sewage Sludge Directive 86/278/EEC that sets out limit values for metals concentrations in sludge and sludge-amended soil. In parallel with increasing quantities of sewage sludge generation, the controversies with respect to the environmental safety of BS are also growing, and the need of the revision of the Sewage Sludge Directive is being currently assessed. The BS application to soil is an irreversible process, thus its temporal weathering transformations should be strongly considered in view of long-term alterations of sorption capacity and binding strength for metals, which may cause metal mobilization. To contribute to clarification of the effect of weathering onto BS capacity for metal binding, a preliminary study was carried out, with use of a unique material – sewage sludge of a known age (0, 5-, 10- and 15 years old) stored in the 24 open shallow lagoons at the Bytom-M (Silesia, Poland) urban wastewater treatment plant. Experimental design comprised assessment of the age-related BS properties exerting a crucial effect on the sorption capacity, such as water content, pH, OM, CECt, exchangeable cations, TOC, functional groups, trace metals (Ni, Zn, Cd, Cu, Cr, Pb) and other elements (Ca, Mg, Fe, Mn), and their binding strength. Also, batch metal sorption experiments were conducted to evaluate age-related changes of BS sorption capacity. Long-term exposure of BS to atmospheric conditions resulted in pH decrease, reduction of OM, TOC, amorphous Fe and declining of HS functional groups involved in metal binding onto humic substances. Since the sorption capacity of BS for trace metals appeared to be up to several orders of magnitude higher than their initial content in BS, no initial metal loads release from BS over time was observed. At the same time, decrease of pH and OM caused a significant decline of weathered BS sorption capacity for metals. This lead to the conclusion that weathering transformations in principle do not affect mobility of metals initially present in BS applied to soil as amendment. On the other hand, at use of BS as sorbents in permeable barriers and protective layers against metal migration, the clear decrease of BS sorption capacity over time may result in metal release and adverse environmental implications.

**RP127 Elucidating a Biosynthetic Pathway for Arsenobetaine in the Terrestrial Fungus *Agaricus bisporus*** M.M. Nearing, Environmental Sciences Group, Royal Military College of Canada, Dept of Chemistry and Chemical Engineering; I. Koch, Environmental Sciences Group, Royal Military College of Canada, Royal Military College of Canada; K.J. Reimer, Environmental Sciences Group, Royal Military College of Canada. Arsenic can be taken up by fungi from contaminated soils and thus subsequently enters food chains. This can cause concern since some arsenic compounds are toxic. Fungi in the class Hymenomycetes collected from arsenic contaminated soils produce fruiting bodies that have been found to contain a variety of arsenic compounds. In many cases arsenobetaine (AB), a non-toxic organoarsenical, comprises the majority of arsenic compounds present in these terrestrial fungi. *Agaricus bisporus* is an example of an edible species of mushroom in which AB is found to be the major arsenic compound. We recently confirmed this was true at ambient concentrations in locally farmed white and brown varieties of *A. bisporus*. The life stage at which the fungus produces AB is still unknown, as is whether the fungus, alternatively, accumulates AB from the surrounding environment. That is, the surrounding microbial community in the soil may produce AB or its precursors and these may be selectively taken up by the fungus. In order to elucidate a potential biosynthetic pathway for AB in the terrestrial fungi the vegetative (mycelium) and reproductive life stages (fruiting bodies) of the fungus *A. bisporus* were cultivated under axenic conditions and exposed to various arsenic compounds including monomethylarsonic acid (MA<sup>V</sup>), dimethylarsinic acid (DMA<sup>V</sup>), and trimethylarsine oxide (TMAO). Mycelium was cultivated in a liquid medium containing 250 µg/L of MA<sup>V</sup>, DMA<sup>V</sup> or TMAO for 21 to 30 days. Arsenic compounds extracted from the mycelium and surrounding growth medium were identified using high-performance liquid chromatography coupled with inductively-coupled plasma mass spectrometry (HPLC-ICP-MS). *A. bisporus* mycelium was capable of accumulating MA<sup>V</sup> (566 – 674 µg/kg dry weight), DMA<sup>V</sup> (426 – 749 µg/kg dry weight) and TMAO (269 – 1159 µg/kg dry weight) from the surrounding growth medium. No biotransformations were observed in any of the treatments indicating that very little arsenic metabolism occurs during this life stage and AB is not produced at the vegetative life stage of *A. bisporus*. Further mycelium exposure experiments using longer exposure periods and other arsenic compounds, for example arsenate (As<sup>V</sup>), are currently being conducted to confirm this finding. The results of the exposure experiments will contribute to the understanding of how AB is synthesized in the terrestrial environment.

**RP128 <sup>68</sup>Zn:<sup>66</sup>Zn Ratio of *L. terrestris* Fed on a Diet of <sup>68</sup>Zn Spiked Oat Bran** K. Linford, Towson Univ, Urban Environmental Biogeochemistry Laboratory; R.E. Casey, Towson Univ, Dept of Chemistry. This study is part of a larger effort to quantify the pool of soil zinc bioavailable to *Lumbricus terrestris*. Enriching *L. terrestris* with <sup>68</sup>Zn can enable us to observe the subsequent decrease of <sup>68</sup>Zn:<sup>66</sup>Zn in *L. terrestris* organ tissues, which previous work has shown is related to soil zinc bioavailability. Previous work in this laboratory evaluated the bioavailable zinc pool in the soil by enriching *L. terrestris* with <sup>68</sup>Zn using <sup>68</sup>Zn-spiked soil. Subsequent experiments showed that the zinc in these zinc-spiked soils becomes unavailable to *L. terrestris* over time (1 year). Thus, Zn spiked food may be a preferable source of enrichment. Studying the effects of zinc dietary exposure was necessary to determine a zinc application rate that would not result in stress to the organism or zinc accumulation in the tissues. Zinc accumulation in the tissues may result in increased zinc tolerance over time. Oat bran was selected as a food item due to its very low background zinc concentration of 11.23 mg/kg and its palatability. An initial experiment showed that feeding oat bran spiked with ZnCl<sub>2</sub> up to 300 mg/kg resulted in no decline in growth rate of the worms. Additionally, no Zn accumulation occurred in the epidermis, gut, or organs of *L. terrestris* upon feeding oat bran spiked with ZnCl<sub>2</sub> (0-500 mg/kg). A feeding rate of 200 mg/kg ZnCl<sub>2</sub> was selected in order to avoid decline in growth. Currently, a kinetic experiment is being performed to determine the amount of time needed to achieve an adequate enrichment of <sup>68</sup>Zn:<sup>66</sup>Zn in *L. terrestris* organ tissues through dietary exposure.

**RP129 Developing a Copper Isotope Ratio Method for Estimating Copper Availability to *Eisenia fetida*** M. Mazzei, Towson Univ; S.M. Lev, D. Ownby, Towson Univ, Urban Environmental Biogeochemistry Laboratory. Determining the extent of uptake and accumulation of micronutrients from the environment by an earthworm such as *Eisenia fetida* is challenging given the organisms ability to regulate internal levels. Trace metals

such as Zn and Cu fall into this category and as a result, make terrestrial risk assessment challenging for sites contaminated by these metals. This is particularly problematic for an element like copper given the plausible risk of trophic transfer as well as toxicity and biomagnification. The use of stable isotopes to trace the exchange (turnover) of metals from the environment into the earthworms' body was demonstrated to be a useful tool for Zn in soil systems. This study extends the method to Cu with three goals: 1) use isotope turnover in the organism as a measure of bioavailability, 2) link toxicity with isotope turnover, and 3) investigate the kinetics of Cu uptake and elimination in *E. fetida*. Cu turnover can be measured using the change in stable copper isotope ratios in the organism after exposure. A 10d copper accumulation and elimination test was performed to understand general turnover rates with respect to two things: our ability to get the enriched <sup>65</sup>Cu isotope into the earthworms and how quickly they would eliminate it once returned to clean soil. All earthworms began in spiked isotope top soil (<sup>65</sup>Cu-enriched) for accumulation and were transferred to non-spiked top soil (<sup>63</sup>Cu-natural abundance) for elimination. This experiment demonstrated that the elimination of <sup>65</sup>Cu occurs within 24h of exposure. A second accumulation and elimination test was performed with two modifications to the above procedure: tighter spaced sampling intervals within a 24h experiment and three concentrations of non-spiked top soil were utilized in the elimination phase. Elimination of <sup>65</sup>Cu occurred within 2h of returning earthworms to natural isotope abundance soil. With estimating bioavailability, that small window of time made measuring bioavailable Cu difficult. The most prominent information gained throughout this endeavor dealt with the kinetics of Cu within *E. fetida*. The isotope ratio method showed that these worms exchange Cu rapidly from an environment where Cu is readily available. This detected physiological exchange would not be possible using an unlabeled soil. Therefore, the drastic isotopic ratio drop does support that Cu was bioavailable from the soil environment, not from internal Cu pools of the earthworm.

**RP130 Geographic Distribution of Heavy Metal Concentrations in Feathers of the Declining Rusty Blackbird** S.T. Edmonds, BioDiversity Research Institute, Wetland Bird Program, Acadia Univ, MSc student; D. Evers, Biodiversity Research Institute; J. Murimboh, Acadia Univ, K.C. Irving Environmental Science Centre; N. O'Driscoll, Acadia Univ, Acadia Univ, Dept of Earth & Environmental Sciences. The Rusty Blackbird has undergone a population decline of over 90% since the 1960s and no consensus has been reached as to the dominant driving factors. Recent studies have shown that mercury concentrations within New England and Maritime Canada are elevated and may potentially act as a negative stressor. We analyzed Rusty Blackbird flight feathers for a suite of heavy metals and selenium from breeding birds in New England, Maritime Canada, and Alaska, and from wintering birds in the southern US to assess the potential for other toxicant stressors and identify those elements that may require additional investigation. Eastern breeding individuals had significantly greater concentrations of cobalt (8x greater, mean = 0.68 ppm), manganese (12x greater, 197 ppm), and lead (1.3x greater, 1.12 ppm) than Alaskan birds, while Alaskan breeding birds had 4x the concentrations of arsenic (0.45 ppm). While few to no studies have examined the effect of these heavy metals on blackbirds, the concentrations observed for manganese (138 ppm) and chromium (5.31 ppm) appear to exceed levels of concern reported for other avian species and warrant further investigation.

**RP131 Realistic Risk: Importance of Site-specific Biota Data for Quantifying BAFs** A. Shortelle, AMEC Environment & Infrastructure; E. Curtis, AMEC Environment & Infrastructure, Project Manager – Ecological Risk Assessment; M. Bystedt, N. Ruberti, T. Glover, AMEC Environment & Infrastructure. A site-specific terrestrial invertebrate bioaccumulation study on arsenic, cadmium, copper, lead, and zinc was conducted for a site in western Georgia using the approach presented in Development and Validation of Bioaccumulation Models for Earthworms (Sample et al., 1998). The bioaccumulation factors (BAFs) and slopes provided in Sample's study are typical of literature based soil-to-biota bioaccumulation factors, but these values have a layer of conservatism, the upper prediction limit, built in for use at sites with no available invertebrate data. The BAFs in Sample (1998) are often used for estimating metal concentrations in biota tissue in food-web modeling in the absence of site-specific biota tissue data. However, the use of the upper predictive limit often leads to overestimation of metal concentrations in biota tissue. The site-specific dataset obtained from the terrestrial invertebrate bioaccumulation study was small and displayed



limited variability. A relationship between soil and tissue concentrations was developed by combining site-specific data with the biota BAFs developed from the larger Sample dataset (1998). Using this approach, a more realistic quantification of risk for ecological receptors was determined than was provided by the sole use of the literature-based BAFs.

**RP132 Risk Assessment and Aquatic Macrophytes: A Method to Test Six Species Individually in a Laboratory Setting** A. Kirkwood, E. Wilson, B. Dinius, J.R. Hoberg, Smithers Viscient, Ecotoxicology. Recently there has been increased concern whether duckweed (*Lemna gibba*) toxicity test results are appropriate for evaluating sensitivity of other aquatic macrophytes. A standardized guideline supported by regulatory agencies is not available for testing aquatic macrophytes. However, an exposure system for this type of test was described by Kubitz and Dohmen (2008) and is presently under inter-laboratory validation with two species: *Myriophyllum spicatum* and *M. aquaticum*. Smithers Viscient validated this method with the above two species as well as four additional aquatic macrophytes: *Ceratophyllum demersum*, *Elodea canadensis*, *Stuckenia pectinata* (formerly *Potamogeton pectinatus*), and *Vallisneria spiralis*. The expansion of this test method to include additional species helps to provide toxicity information across four different families. Shoot dry weight is the test endpoint most commonly used for evaluation of toxicity; an increase of  $\geq 2X$  in dry weight over 14 days is typical for each species. Therefore, test results are appropriate for species sensitivity distribution in the risk assessment of chemicals. This poster presents a description of the exposure method and control growth data for each species mentioned above.

**RP133 A Proposed OECD Test Guideline for the Submerged, Sediment-rooted Macrophyte, *Myriophyllum*** J. Davies, Syngenta, Environmental Safety; P. Dohmen, BASF AG, Landw. Versuchsanstalt, APD/RO, BASF SE, Ecotoxicology dept; G.H. Arts, Alterra Wageningen Univ and Research Centre, Centre for Water and Climate, Environmental Risk Assessment. Under current EU pesticide regulation, regulatory tests are required for the aquatic macrophyte, *Lemna*, and two algal species for herbicides. In 2008, the SETAC – AMRAP (Aquatic Macrophyte Risk Assessment for Pesticides) workshop identified the potential need for regulatory tests with submerged, rooted macrophyte species in cases where root uptake from sediment is considered an issue or where the sensitivity of standard algae and *Lemna* species is believed not to be representative of other macrophyte species. In light of existing experience, *Myriophyllum* species were selected as the potential second macrophyte test species and a work group was established to develop an appropriate test method. Over the past 3 years, the suitability of the proposed method has been evaluated in a series of ring-tests performed in >12 laboratories. In 2011, the method was proposed and accepted as an OECD test guideline project and is currently undergoing a further phase of ring-testing. Progress to date will be described in this presentation.

**RP134 Evaluation of Biomarkers in *Spartina alterniflora* from Galveston Bay, Texas, Related to Ambient Environmental and Heavy Metal Stress** C.L. Howard, Univ of Houston-Clear Lake, Dept of Biological & Environmental Sciences; C. Gauthier, J. Borski, Univ of Houston Clear Lake, Biological and Environmental Science. Tidal wetlands are among the most productive ecosystems in North America. In Gulf of Mexico estuaries, these wetlands are dominated by the perennial halophyte *Spartina alterniflora*. *S. alterniflora* exhibits multiple height morphs and varying physiological characteristics, which have been shown to exert a significant impact on the overall function of marsh ecosystems. For the past six years, we have been studying stress biomarkers (peroxidase, catalase, glutathione reductase and protein), ecotypes and sediment heavy metal distribution in 15 *S. alterniflora* marshes bordering Galveston Bay, Texas. The populations at each site vary in history (reference, created, pollutant-impacted), salinity and tidal regime, dominant growth form, productivity and sediment heavy metal content. The objective of the present work was to assemble the data from several years' collections and determine patterns in biomarkers and growth forms that can be related to heavy metal exposure, separate from normal environmental stressors. A total of 720 individual plants were collected from the 15 populations over a 3-year period; field parameters, growth metrics and chlorophyll (SPAD-1500) data were recorded on site. Shoot peroxidase (POD), catalase (CAT), and glutathione reductase (GSR) activities and protein (PTN) concentration were determined in the lab using standard spectrophotometric assays. Shoots and sediments were acid digested and

heavy metal concentrations were measured by ICP. ANOVA and PCA were used to determine that qualitative and quantitative differences in stress biomarkers, growth metrics and productivity are related qualitatively and quantitatively to heavy metal exposure in the 15 populations.

**RP135 Monitoring Diurnal Variations of pH and Oxygen as Indicators of Macrophyte and Algae Productivity in Mesocosms** P. Ebke, L. Doering, MESOCOSM GmbH; U. Hommen, Fraunhofer IME. Mesocosms studies are an established tool to assess the impact of herbicides on primary producers (algae & macrophytes) in aquatic ecosystems. Additional to the endpoints surface coverage, biomass or growth rate of macrophyte as well as abundances and community structure of phytoplankton and periphyton, the OECD Guidance Document on Simulated Freshwater Lentic Field Tests recommends also the measurement of primary productivity e.g., by monitoring diurnal fluctuations of the oxygen concentration in the water. However, these diurnal oxygen measurements require high efforts, i. e. due to the need of values taken before sunset to get the lowest diurnal oxygen level. Within an ongoing research project on effects of multiple stressors on aquatic organisms and ecosystems an automatic measurement system is developed for the use in outdoor mesocosms, which is also applicable in field monitoring as well as laboratory studies. The system provides online pH- and oxygen data in combination with phytoplankton analysis based on delayed fluorescence spectroscopy for pigment analysis of the water samples. The system has been tested in different mesocosms over several weeks yet. The quasi-continuous pH and oxygen data provide information on total daily oxygen consumption and production. In addition, the delayed fluorescence spectroscopy analysis provides information on the phytoplankton pigment concentration (chlorophyll a) and community composition (with respect to five algae groups differentiated according to their pigment composition). Thus, toxic effects can be detected on the level of the phytoplankton community as important structural endpoint and on the level of total primary productivity as functional endpoint. The poster will present first results revealed with this online measurement system and will demonstrate the added value for the assessment of chemical effects on primary producers in aquatic mesocosms.

**RP136 Rapid Injury Assessment of Submerged Aquatic Vegetation Communities in the Northern Gulf of Mexico Following the Deepwater Horizon Accident** R. Burdge, M. Barth, A. Meyers, Cardno Entrix. In response to the Deepwater Horizon accident, federal and state trustees and BP scientists are cooperatively assessing injury to the submerged aquatic vegetation (SAV) communities as part of the initial phase of the Natural Resource Damage Assessment (NRDA). The cooperative team conducted a rapid habitat assessment survey between August 24 and September 16, 2010 that examined the health and environmental conditions of SAV communities potentially exposed to MC-252 oil. Based on oiling exposure trajectories and shoreline cleanup assessment technique (SCAT) information, 72 sample sites at risk for exposure in the Northern Gulf of Mexico, from Louisiana to Florida, were identified and sampled. Rapid habitat assessments consisted of visual surveys of the health and condition of SAV habitats, including percentage of SAV cover, presence of new SAV growth, and incidences of oiling. Additionally, sediment, water, and invertebrate and plant tissue samples were collected for chemical analysis, fingerprinting, polycyclic aromatic hydrocarbon (PAH) concentrations, and presence of dispersants. Overall health and environmental conditions of SAV beds surveyed were comparable to pre-impact assessments, but additional habitat evaluations are necessary before determinations can be made as to the extent of potential injury or restoration potential for SAV habitats impacted by the accident. This poster will describe methods used and provide a visual examination of data collected during the rapid habitat assessment survey.

**RP137 A Novel Weight of Evidence Approach to Distinguish Between Toxicological Impairment and Nutrient Enrichment Impacts in an Oligotrophic Lake** R. Stevenson, Golder Associates; G. MacDonald, Diavik Diamond Mine Inc.; P. Chapman, Golder Associates. Diavik Diamond Mine is located on Lac de Gras, an oligotrophic lake, in NWT, Canada. Annually, the Mine conducts an Aquatic Effects Monitoring Program (AEMP) to examine the impacts of Mine operations on the aquatic resources of Lac de Gras. A risk-based weight of evidence (WOE) framework has been developed to integrate the AEMP findings and evaluate two competing impact hypotheses for Lac de Gras. The Toxicological Impairment Hypothesis postulates that toxicity to aquatic organisms could occur due to metals released to Lac de Gras. Alternatively, the Nutrient Enrichment Hypothesis

postulates that eutrophication could occur due to the release of nutrients to Lac de Gras. For each hypothesis, the WOE framework integrates endpoints representing nutrient/contaminant exposure and biological response with a priori weighting factors, direction weighting factors and a posteriori weighting factors to provide a categorical ranking of the Evidence of Impact on lake productivity, the benthic invertebrate community, and the fish community. A higher rank represents a greater strength of support for a particular hypothesis. The weighting factors quantify professional judgement with respect to the ability of specific endpoints to indicate impacts, how much a particular biological response supports each hypothesis, and the degree of coherence/causality in endpoint responses. Thus, the process for arriving at AEMP conclusions is communicated in a transparent fashion and the influence of specific judgements on study findings can be evaluated. This approach is seen as a significant augmentation to WOE approaches that have typically only addressed contaminant-related toxicological impairment. This is particularly important for Diavik Diamond Mine, where mild nutrient enrichment, rather than toxicological impairment, is expected to be the primary impact on Lac de Gras. Review of the WOE application over the past 4 years can inform refinement of the AEMP by highlighting the main drivers of annual AEMP conclusions. The WOE framework will be presented along with recommendations and lessons learned.

**RP138 An Aquatic and Terrestrial Hazard Evaluation of Petroleum Coke** J.P. Swigert, EcoTox Assessments, LLC; C. Lee, ExxonMobil Biomedical Sciences, Inc.; D.C. Wong, Shell Oil Company – Shell Health; P. Podhasky, American Petroleum Institute. Aquatic and terrestrial ecotoxicity tests were conducted as part of the USEPA High Production Volume (HPV) chemicals programs in order to assess the hazard of petroleum coke (CAS 64741-79-3) to representative aquatic organisms and terrestrial soil-dwelling invertebrates and vascular plants. Acute toxicity test species included a fish (*Pimephales promelas*), an aquatic invertebrate (*Daphnia magna*), and an alga (*Pseudokirchneriella subcapitata*). Aquatic exposure solutions were prepared as water accommodated fractions (WAFs) and attempts were made to analytically quantify 19 polycyclic aromatic hydrocarbon compounds (PAH) and seven inorganic constituents (Fe, S, As, Se, Cu, Ni, and V) of petroleum coke in the WAF solutions. All results of these analyses were below minimum detection limits. Petroleum coke used in aquatic tests had a mean particle size of 2 mm. This facilitated segregation of the solid particles from the WAF and avoided potential physical entrapment of the coke in the respiratory membranes of the test organisms. In all aquatic tests, the 96-h or 48-h LL/EL50 values were greater than the maximum WAF loading rate of 1000 mg coke/L. Terrestrial toxicity tests were conducted with the earthworm (*Eisenia fetida*) in a 14-d exposure, and three vascular plant species, corn (*Zea mays*), radish (*Raphanus sativus*), and soybean (*Glycine max*) each in a 21-d growth test. Terrestrial tests were run with a powdered form of petroleum coke added directly to the soil to maximize contact with earthworms and plant roots. The mean particle size of the powdered coke was 3.3 µm. The earthworm test showed a 14-d EC50 of >1000 mg coke/Kg soil. Terrestrial plant tests resulted in 21-d LC50 values of >1000 mg coke/Kg soil for all species. Analysis of test soil for PAHs and the inorganic components associated with coke was not done based on calculations that none of the constituents would exist above minimum detection limits or soil background levels. Under the conditions of these tests, the results indicate a low potential for petroleum coke to cause adverse effects in the aquatic or terrestrial environment.

**RP139 Apparent Algal Toxicity of a Poly-anionic Polymer Mitigated by the Stoichiometric Addition of Calcium** J. Carbone, G.A. Hazelton, Dow Chemical Company, Toxicology, Environmental Research and Consulting; J. Manna, Dow Chemical Company, Home and Personal Care; K.R. Lampe, Dow Chemical Company, Toxicology, Environmental Research and Consulting; S. Dyer, The Procter & Gamble Company, Central Product Safety, The Procter & Gamble Company, Miami Valley Innovation Center; K. Softcheck, Smithers Viscient. Dow has developed a poly-anionic polymer for use as a substitute for phosphates in automatic dish detergents. The polymer functions as a calcium chelator and soil re-deposition inhibitor. The ecotoxicity of the polymer was tested in acute Fathead Minnow and *Daphnia magna* studies. The polymer was also tested with the green alga *Pseudokirchneriella subcapitata* employing the three tiered testing approach from Nabholz, et al., 1993. Test 1 used standard Algal Assay Procedure (AAP) medium (hardness of approximately 15 mg/L as CaCO<sub>3</sub>). Test 2 used standard AAP medium supplemented with calcium (Ca<sup>2+</sup> as CaCl<sub>2</sub> • 2H<sub>2</sub>O) on a stoichiometric basis at a 1:2 mole ratio with the carboxylate groups of

the test substance. Test 3 used AAP medium which had been adjusted to a hardness of 150 mg/L as CaCO<sub>3</sub>, using a 2:1 calcium:magnesium addition. The purpose of these tests were to define the toxicity of the test substance under standard algal test conditions in a low hardness medium (Test 1), after the active carboxyl groups were bound with calcium (Test 2) and within algal medium adjusted to moderately hard water conditions (Test 3). Test 3 was also used in conjunction with the *Daphnia magna* and Fathead Minnow studies in order to determine species sensitivity. The results of the Fathead Minnow, *D. magna* and *Pseudokirchneriella subcapitata* toxicity studies with culture media supplemented with CaCO<sub>3</sub> at 150 mg/L indicated that *Pseudokirchneriella subcapitata* was the most sensitive species. Based on cell density the 96-hour EC50 was 130 mg active ingredient as polymer, whereas for fish and *D. magna* the EC50's were > 950 and 710 mg a.i./L, respectively. The EC50 of Test 1, with the non supplemented AAP algal medium equaled 4.2 mg a.i./L. For test 2 with the standard AAP medium with calcium added on a stoichiometric basis, the EC50 was found to be >140 mg a.i./L. The results indicated that the apparent algal toxicity of a poly-anionic polymer can be mitigated via the stoichiometric addition of calcium. The algal toxicity endpoints for Test 1 and Test 2 were employed in the USEPA Sustainable Futures risk assessment paradigm. Results of the risk assessment demonstrate a negligible potential for adverse environmental effects.

**RP140 Application of Unique Test Design in Determining the Chronic Toxicity of Boron to *Lumbriculus variegatus* in Whole-sediment Tests** W. Hall, ENVIRON International Corporation, Ecotoxicology, ENVIRON; M. Harrass, Rio Tinto Minerals; R. Lockwood, ENVIRON. Presentation Type: Poster Preferred Track: Aquatic Toxicology and Ecology Session: Aquatic Toxicology and Ecology General Abstract Title: Application of a Unique Test Design in Determining the Chronic Toxicity of Boron to *Lumbriculus variegatus* in Whole-Sediment Exposures Authors: Scott Hall, Rick Lockwood – ENVIRON International, Nashville, TN Michael C. Harrass, Ph.D. – Rio Tinto Minerals, Hoffman Estates, IL Abstract: The chronic toxicity of boron (as boric acid) to the freshwater benthic worm *Lumbriculus variegatus* was evaluated in a 28-day study with spiked natural sediment in a flow-through test system. Following the OECD Method 225 procedure, sediments were spiked in a range-finding test. Approximately 20% of the overlying water was replaced per day in the flow-through test system. The initial tests showed rapid dissolution and removal of the water-soluble boric acid from the sediments. To achieve a more constant exposure in the definitive test, boric acid was added to the replacement waters in concentrations nominally equal to the whole-sediment values. This approach resulted in excellent agreement (generally within 10 percent) between nominal and measured total boron concentrations in each medium (overlying water, sediment pore-water, and whole sediment) throughout the 28-d test. Chronic endpoints (IC25) for survival and growth were 102.4 and 235.5 mg B/kg dry sediment, respectively. The modified procedure proved highly reliable in maintaining overlying water quality conditions (dissolved oxygen, pH, and ammonia) and whole-sediment boron concentrations during the 28-d study.

**RP141 Aquatic Hazard Characterization of Commercial Naphthenic Acids from Crude Oil Origin** J.P. Swigert, EcoTox Assessments, LLC; D.C. Wong, Shell Oil Company – Shell Health; C. Lee, ExxonMobil Biomedical Sciences, Inc.; P.M. Fedorak, Univ Alberta; P. Podhasky, American Petroleum Institute. Naphthenic acids (NAs) are naturally-occurring carboxylic acids (formula C<sub>n</sub>H<sub>2n+2</sub>O<sub>2</sub>) present in crude oil and oil sands bitumen. Refining of crude oil concentrates NAs in the middle distillate streams, from which they are removed during the finishing of the fuel products. Extraction of bitumen from oil sands generates oil sands process-affected wastewaters (OSPW) that also contain NAs. To quantify the acute aquatic toxicity of NAs, studies with representatives from three trophic levels (fish, invertebrate, algae) were performed. Test exposures were based on water accommodated fractions (WAF) of crude oil refined commercial NAs product and included GC-MS characterization and quantification of NAs. Results showed fish (*Pimephales promelas*) to be more sensitive to NAs exposures than invertebrates (*Daphnia magna*) or algae (*Pseudokirchneriella subcapitata*). Acute endpoints (LL/EL50) for fish, invertebrates, and algae based on WAF loading of total NAs were 9.0 mg/L, 24 mg/L, and 25 mg/L, respectively. Endpoints (LC/EC50) based on mean measured concentrations were 5.6 mg/L, 20 mg/L, and 18 mg/L, respectively. These new data establish the acute aquatic toxicity of a commercial NAs preparation for standard test organism endpoints not previously reported. GC-MS characterization showed that the test substance and the WAF-soluble NAs were composed

mostly of 1-ring and 2-ring isomeric structures with carbon numbers most commonly observed in the 12 or 13 range. Acyclic acids were the third most prevalent acids followed by NAs having 3-, 4-, and 5-ring structures. Commercial NAs that was used in these tests differ from those found in OSPW in that the NAs in OSPW are reported to be predominantly 2- and 3-ring compounds.

**RP142 Assessing Water Quality Impairment by Quantifying Metal Concentrations in Aquatic Invertebrates in the Absence of Water Data** J.L. Ullman, Univ of Florida, Agricultural & Biological Engineering; S.J. DeBano, D. Wooster, Oregon State Univ, Dept of Fisheries and Wildlife; D. Horneck, Oregon State Univ; X. Lu, Washington State Univ; J. Snyder, Oregon State Univ, Entomology Program. The Umatilla River Basin in eastern Oregon is a highly impacted watershed that receives inputs from agriculture and municipal sources. In addition to other pollutants, metals are a concern as segments of the watershed have been declared water quality limited under Section 303(d) of the Clean Water Act for iron and manganese. However, water quality data for metals is essentially nonexistent due to resource limitations. This study quantified metal accumulation in aquatic invertebrates to examine potential metal impacts on biota in the absence of water data. Crayfish and stoneflies were collected at fifteen locations along the river, which included sites selected to delineate inputs associated with major tributaries impacted by agriculture and two wastewater treatment plants, as well as a reference site in the wilderness area of the upper watershed. Crayfish and stonefly tissues were digested and analyzed for heavy metals using inductively-coupled plasma mass spectrometry (ICP-MS). The pollutants considered here include arsenic, cadmium, chromium, copper, lead, mercury, silver and zinc. Despite numerous potential metal inputs throughout the basin, analysis of the invertebrates revealed no clear overall trends in bioaccumulation along the river. The reference site frequently exhibited metal levels as high or higher than the "impacted" sites downstream, indicating natural inputs. While copper concentrations in stoneflies generally increased from means of below 10 µg/g to about 30 µg/g when progressing downstream, zinc accumulation in crayfish actually decreased from 74.5 to 41.9 µg/g between the upper-most and lowest sites and arsenic showed a significant linear decrease moving downstream. The concentrations for all elements were comparable to other studies in similar watersheds except for chromium, which reached levels of 21.06 µg/g (comparable rivers did not exceed 2.5 µg/g). Although wastewater treatment plants are often sources of metal pollutants and agricultural fertilizers are known to introduce cadmium into the environment, no significant increased concentrations were observed for any of the metals in relationship to known inputs from these sources. This study highlights the need to conduct a robust assessment of water quality impacts on aquatic habitat before mandating remediation activities, especially when water quality data are lacking.

**RP143 Chloride Toxicity in an Urban Missouri Stream** C. Cole-Neal, Central Methodist Univ; A.L. Allert, USGS – Biological Resources Division, Columbia Environmental Research Center, USGS. – Columbia Environmental Research Center, Biological Resources Division; J. Fairchild, USGS – Biological Resources Division, Columbia Environmental Research Center. Urbanization leads to increases in impervious surfaces such as rooftops, parking lots, and roads. Surface run-off transports increased levels of sediment and contaminants to streams due to accelerated water flow associated with storm-water runoff. In northern climes impervious surfaces are frequently treated during winter with deicers such as sodium chloride (NaCl) and calcium chloride (CaCl<sub>2</sub>) for public safety. Long-term effects of increased chloride concentrations in subsequent storm-water runoff can cause a wide range of adverse effects in aquatic environments such as loss of sensitive species and changes in community structure, diversity, and productivity. We conducted a study of winter storm-water runoff in Columbia Missouri to evaluate the chronic effects of road-salt based chloride on survival and reproduction of *Ceriodaphnia dubia*. Water was collected from seven mainstem and tributary sites of Hinkson Creek. Chloride concentrations at two sites exceeded the US chronic water quality criterion of 235 mg/L chloride during the 7-day *Ceriodaphnia dubia* toxicity test. The highest measured concentration (1252 mg/L chloride) was about the concentration of published acute 48-hr LC50 toxicity values for NaCl (1960 mg/L) and chloride (1189 mg/L). Mortality was significantly higher and total reproduction was significantly lower in *C. dubia* in test water collected from this site during the laboratory study. Comparison of the response distributions of *C. dubia* exposed to field-collected stream samples and a separate single-chemical

(NaCl) study gave similar results, indicating that chloride was the likely toxicant of concern. These data indicate current deicing practices in the Hinkson Creek watershed are likely having adverse effects on aquatic life in receiving waters and that adaptive management procedures including use of alternative de-icers or GIS-based mapping of geomorphological conditions and salt applications could protect aquatic life in this urban stream.

**RP144 Combined Toxic Effects of Surfactants and PPCPs on Aquatic Organisms** K. Kagota, Univ of Tokushima, faculty of integrated arts and science. Surfactants have been reported to increase the fluidity of cell membrane molecules, which may be resulted in the change in membrane permeability of the chemical compounds with different physical-chemical properties. Most of pharmaceuticals and personal care products (PPCPs) have been designed to have a certain physiological activity and could exert adverse effects on aquatic organisms at relatively low concentration. Numerous researchers have reported the ecotoxicity of individual surfactants and PPCPs on aquatic organisms, but their combined effect remained unknown. The objective of this study is to evaluate the combined effects of surfactants and PPCPs by acute, and sub-chronic toxicity tests, and several biomarkers using Japanese medaka (*Oryzias latipes*), zebrafish (*Danio rerio*) and *Daphnia magna*. An anionic surfactant C12-LAS was used as a model surfactant and combined effects with major pharmaceuticals such as propranolol, mefenamic acid, and triclosan were examined. Combined toxicity tests were carried out with the concentration addition mode based on LC50, EC50 and EC10 values of the individual compounds. Additive effects were found for the combination of C12-LAS and mefenamic acid for all the tested organisms. Whereas nearly the additive effects were found for the combination of C12-LAS and propranolol in short term toxicity test using *Danio rerio*, slight synergistic effects were evident in acute toxicity test using *Oryzias latipes*. In contrast, additive effects were found for the combination of C12-LAS and triclosan in acute toxicity test using *Oryzias latipes*, but antagonistic effects were evident in the sub-chronic toxicity test using *Danio rerio*. The differences in fish species, membrane formulations, and physiological effects (mode of actions) could cause the difference in combined effects in acute tests using juvenile medaka fish and sub-chronic tests using embryo-larvals of zebrafish.

**RP145 Constructed Wetland Treatment Systems for Energy-derived Produced Waters: Treating Selenium for Surface Water Discharge** L. Rodgers, Clemson Univ, Forestry and Natural Resources; J. Castle, Clemson Univ, Environmental Engineering and Earth Sciences; M. Spacil, Diamond V; C. Ritter, Clemson Univ. Energy derived produced waters (EDPWs) are generated in relatively large volumes (~3.3 million m<sup>3</sup> annually) from a variety of sources, and these waters may contain constituents from source formations as well as process chemicals that were added. Selenium (Se) is a common constituent requiring treatment prior to discharge or beneficial reuse. An effective and reliable approach is needed to treat Se in EDPWs to meet stringent water discharge limits established under the National Pollutant Discharge Elimination System (NPDES) and the Clean Water Act. This study focused on an effluent containing Se that was produced as a byproduct of petroleum refining. Pilot-scale constructed wetland treatment systems (CWTSs) were designed and built at Clemson Univ to evaluate removal of Se from simulated refinery effluent (SRE). Specific objectives were to: 1) chemically and physically characterize a specific petroleum refinery effluent for simulation and confirm Se as a constituent of concern, 2) design and conduct bench-scale experiments to measure Se removal in response to organic carbon additions, 3) design and build a pilot-scale CWTS using information from the bench-scale experiments, and 4) measure performance of the pilot-scale CWTS in terms of rate and extent of Se removal in response to organic carbon additions. The treatment goal (i.e., Se removal) for this study was to decrease the Se concentration in SRE from ~50 µg/L to less than 5 µg/L. Samples were collected from the inflow and the outflows of each wetland cell in the pilot-scale CWTS. In order to determine rates and extents of Se removal and factors that may influence performance, parameters measured included elemental analysis of Se, dissolved oxygen concentration, conductivity, pH, alkalinity, hardness, and temperature. With organic carbon additions to the SRE inflow, outflow Se concentrations ranged from 3.4 to 9.8 µg/L, depending on the experimental treatment. This pilot-scale study illustrates that CWTSs can enhance Se removal from a SRE and required performance (i.e., removal of Se to < 5 µg/L) can be achieved to meet stringent discharge limits.



**RP146 Continued Investigation into Potential Endocrine Disruption in Cacapon River Populations of the Freshwater Mussel *Elliptio complanata*** D. Sovic, Ohio State Univ; R. Lanno, Ohio State Univ, Dept of Evolution, Ecology, and Organismal Biology. A high prevalence of intersex condition, or the appearance of oocytes in the testis, found in male small-mouth bass collected from populations throughout the eastern panhandle of West Virginia has provided evidence of the possible endocrine disruptive potential of surface waters in this area. To investigate the potential that other aquatic species are being similarly affected, a study has been undertaken to evaluate possible endocrine disruption in two populations of the freshwater mussel, *Elliptio complanata*, located in West Virginia's Cacapon River, in areas of both high and low agricultural activity. Data suggest significant differences in sex ratios between the two populations, as mussels sampled in close proximity to high levels of agricultural activity and in forested regions exhibited approximately 2.5:1 and 1.3:1 female-to-male ratios, respectively. Additionally, histological evidence of gamete developmental asynchrony, particularly within female mussels of the agriculturally associated population has been observed. Rates of viable and nonviable ova, as well as mean ova diameters from biopsies collected in Spring 2010 support differential reproductive development between populations. Changes in total mass, as well as shell length, width, and height between Autumn 2009 and Autumn 2010 indicate positive growth in only that population associated with low agricultural activity. Groups from each population were relocated between sites in Autumn 2010. These individuals will be recaptured and reevaluated to determine whether any differences observed are population or site specific. Sediment and surface water extracts collected periodically between Summer 2010 and Fall 2011 will be tested for total estrogenicity with the recombinant yeast-based estrogen assay (YES). In addition, the potential for utilizing mussel hemolymph as an indicator of exposure to estrogenic compounds will be explored.

**RP147 Designing Constructed Wetland Treatments Systems for Ammonia in Produced Water** D. Beebe, Y. Song, J. Castle, Clemson Univ, Environmental Engineering and Earth Sciences; J. Rodgers, Clemson Univ, Forestry and Natural Resources. Constructed wetland treatment systems (CWTSs) can be engineered to create the biogeochemical conditions needed to promote pathways for treatment of constituents of concern in impaired waters so that they may be used beneficially. In this study, pilot-scale CWTSs containing *Typha latifolia* (broadleaf cattail) were designed to remove ammonia from simulated post-reverse osmosis produced water to concentrations required to meet stringent discharge or beneficial use requirements (< 1.2 mg/L as N). The objectives of this research were to (1) identify the biogeochemical conditions required for microbial nitrification and denitrification, (2) construct a pilot-scale CWTS with amendments designed to provide the required treatment conditions, (3) determine ammonia removal performance of the amended CWTS, and (4) compare ammonia removal of the amended CWTS with a CWTS containing no amendments. Based on biogeochemical conditions required for microbial nitrification and denitrification (e.g., dissolved oxygen, pH, and alkalinity) a *Typha* pilot-scale CWTS was designed and constructed with amendments including mechanical aeration to increase dissolved oxygen concentrations, metered sucrose additions to serve as a source of electron donors, crushed oyster shell additions to stabilize pH and alkalinity, and an 8-day hydraulic retention time (HRT). Ammonia removal and related chemical parameters from the amended CWTS were compared with an unamended *Typha* CWTS operating on a 4-day HRT. Results indicate that the amended CWTS was capable of lowering ammonia-N concentrations from approximately 20 mg/L to non-detectable levels (< 0.1 mg/L) versus 12.6 mg/L for the unamended CWTS. Calculated rate coefficients for the amended CWTS ranged from 1.35 to 1.39 d<sup>-1</sup> versus 0.071 to 0.111 d<sup>-1</sup> for the unamended CWTS. Measured explanatory parameters related to ammonia removal (e.g., dissolved oxygen, pH, and alkalinity) were within the required range for the amended CWTS indicating that the selected amendments were capable of promoting the biogeochemical conditions needed for ammonia removal. Pilot-scale *Typha* CWTSs designed to promote microbial nitrification and denitrification pathways can successfully treat ammonia to concentrations required for reuse or discharge.

**RP148 Disruption of E2-induced Non-genomic Signaling by Combinations of Bisphenol A and Nonylphenol** R. Vinas, Univ of Texas Medical Branch – Galveston, Dept of Biochemistry & Molecular Biology, Univ of Texas Medical Branch – Galveston, Pharmacology & Toxicology; C.S.

Watson, Univ of Texas. Medical Branch – Galveston, Dept of Biochemistry & Molecular Biology. Xenoestrogens (XEs) can mimic or antagonize the effects of physiological estrogens by binding to membrane bound estrogen receptors (mER $\alpha$ , mER $\beta$ , GPER/GPR30). Detected in appreciable amounts in human serum and urine (nM range), 4-nonylphenol (NP) and bisphenol A (BPA) are environmental pollutants known for their estrogenic effects. Previous studies from our lab in pituitary cells have demonstrated that these steroid-mimicking compounds potently and rapidly exert their effects via activation of non-genomic signaling pathways (e.g. mitogen activated protein kinases, Ca<sup>2+</sup> firing, prolactin release). This study aimed to characterize the combined effect of XEs (BPA & NP) with a physiological estrogen, estradiol (E2), on kinase signaling pathways. Using the GH3/B6/F10 rat pituitary cell line we quantified phosphorylations of estrogen-induced extracellular signal-regulated kinases (ERKs) by a quantitative high-throughput plate immuno-assay. BPA and NP both activated ERKs in a dose-(fM-nM) and time-dependent (2.5-60 min) manner. E2 [1nM] was used as a standard for each experiment. Low XE concentrations (sub-pM) enhanced, while higher concentrations (pM-nM) diminished ERK activation, resulting in non-monotonic dose-responses. Combination of XEs with E2 [1nM] did not result in the expected additive effect, but instead attenuated ERK activation. BPA or NP at two different concentrations (10fM and 10nM) in combination with E2 [1nM] activated ERKs throughout the 60 min time course study, but caused temporal response patterns different from E2 [1nM] alone. Cell exposure to the combination of BPA [10fM], NP [10nM], and E2 [1nM] resulted in a drastic inhibition of ERK activation, but simultaneously activated c-Jun-N-terminal kinase (JNK) over 60 min. In conclusion, combinations of BPA and NP at environmentally relevant concentrations disrupt E2-induced non-genomic signaling. Risk assessment of chemical mixtures should therefore be more carefully addressed with respect to concentration and time, as these results suggest that XEs disrupt the normal functions of estrogens in the pituitary, which may affect functions during key life phases such as development, reproductive cycling, and pregnancy.

**RP149 Ecotoxicity Issues Encountered in Complying with REACH Requirements** K. Mierau, ENVIRON International Corp.; S. Deacon, ENVIRON UK Ltd. The European REACH (Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals) Regulation requires all manufacturers and importers of chemicals to identify and manage risks associated with the substances they manufacture. As part of the hazard assessment, predicted no-effect concentrations (PNECs) are estimated for long- or short-term exposure within an environmental compartment, (e.g., freshwater, marine, sediment, soil, etc.). PNECs are derived from ecotoxicity data using extrapolation methods such as species sensitivity distribution or assessment factor approaches. Grouping, read-across, and equilibrium partitioning methods can also be employed where data gaps exist. Although REACH Guidance describes the process for PNEC development, alternative approaches may be needed for complex chemicals or non-standard issues, such as endocrine disrupting properties, hydrocarbon compounds with moderate solubility, nanoparticles, and speciation. Additional issues may arise for those constituents that simply have a lack of reliable ecotoxicity data available. This poster presentation will discuss some of the ecotoxicity issues that were encountered during the REACH registration dossier preparations for multiple chemicals in support of the November 2010 deadline.

**RP150 Ecotoxicological Assessment of the Clinch River, Virginia, to Determine Major Contributors of Trace Metals and Implications for Unionid Impairment** B.S. Echols, D.S. Cherry, Virginia Tech, Dept of Biological Sciences. Analysis of sediments and interstitial water (porewater) indicate higher concentrations of trace metals in samples from sites located above both a power plant (CRP) and Dumps Creek mining influences. The furthest upstream sampling site located near Pounding Mill, Virginia (CR-PM) had higher concentrations of aluminum (2,250.9 mg/kg), copper (5.9 mg/kg) and iron (12,322.6 mg/kg) in sediment compared to samples collected directly below the Dumps Creek confluence (site CR-2). Similar results were obtained from bioaccumulation in situ tests with the Asian clam (*Corbicula fluminea*) in 2009. Aluminum (7.81 mg/kg), Fe (48.25 mg/kg) and Zn (7.69 mg/kg) were accumulated in higher concentrations at CR-PM site than CR-2. However, the site located below the CRP effluent discharges (CR-3L) on the left bank had substantially higher concentrations of Al (14.19 mg/kg), Cu (6.78 mg/kg), Fe (88.78 mg/kg) and Zn (7.75 mg/kg) than both CR-PM and samples collected directly opposite of this site at CR-3R. Bioconcentration Factors (BCF) calculated for clam tissues and

portewater metals (Al, Fe, Cu, Zn) were higher for samples downstream of the CRP effluent than right bank samples, which are primarily influenced by Dumps Creek. For example, BCF values were highest for Cu (2,196.7) at CR-3L, but substantially lower (798.8) at CR-3R. Benthic invertebrate surveys below the CRP also indicate a shift in taxa composition below the CRP, further indicating a substantial influence of discharges on river biota. Multiple sources of impact, including unidentified sources upstream, active coal mining in the Dumps Creek subwatershed and discharges from the CRP power plant are contributing to continual metal contamination in the Clinch River. This chronic exposure may be contributing to depressed unionid recruitment at downstream reaches. Additionally, these stressors in addition to past insults have caused biological impairment and unionid decline in the Clinch River and ultimately have affected the resiliency of the river.

**RP151 Effects of a Model 3 $\beta$ -Hydroxysteroid Dehydrogenase Inhibitor, Trilostane, on Reproductive Endocrine Function in the Fathead Minnow** K.M. Jensen, USEPA, Mid-Continent Ecology Division; J.E. Cavallin, E.J. Durhan, M.D. Kahl, E.A. Makynen, USEPA; D. Martinovic-Weigelt, Univ of St. Thomas, Dept of Biology, USEPA, Mid-Continent Ecology Division, Mid-Continent Ecology Division, Univ of St. Thomas, Biology; L. Wehmas, USEPA; D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division. Inhibition of enzymes involved in the synthesis of sex steroids can substantially impact developmental and reproductive processes controlled by the hypothalamic-pituitary-gonadal (HPG) axis. A key steroidogenic enzyme that has received little attention from a toxicological perspective is 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD). A number of environmental contaminants and plant flavonoid compounds have been shown to inhibit 3 $\beta$ -HSD activity, indicating that this may be an environmentally-relevant mechanism of HPG axis disruption. In these studies we exposed reproductively-mature fathead minnows (*Pimephales promelas*) to the model 3 $\beta$ -HSD inhibitor trilostane at test concentrations ranging from 50 to 1500  $\mu$ g/L for up to 21 d. Shorter-term time-course exposures included a clean water recovery phase. Exposure to trilostane caused a significant reduction in fecundity. Plasma concentrations of 17 $\beta$ -estradiol (E2) in females were rapidly depressed within hours of exposure and were accompanied by decreases in plasma concentrations of the estrogen-responsive protein vitellogenin (VTG). Decreased vitellogenesis in vivo was consistent with inhibition of in vitro production of E2 by fathead minnow ovaries. Plasma E2 and VTG concentrations quickly returned to control levels during the recovery phase. Up-regulation of ovarian expression of gene products for follicle stimulating hormone receptor (*fshr*), and the cytochrome P450-based enzyme aromatase suggested active compensation in trilostane-exposed animals. The effects on HPG function in exposed males were less pronounced, however, trilostane significantly increased gonadosomatic index and, similar to females, caused up-regulation of gonadal *fshr*. The results of these studies support the hypothesis that 3 $\beta$ -HSD inhibition can cause reproductive dysfunction in fish. The data obtained from the time-course studies provide additional insights as to direct impacts, compensatory responses, and recovery from effects associated with 3 $\beta$ -HSD perturbation. The return of E2 and VTG to control levels in fish removed from the trilostane exposure demonstrates the highly dynamic, adaptive nature of the HPG axis in response to stressors. This information is important to the design and interpretation of approaches for assessing the occurrence and effects of HPG-active chemicals both in the lab and field. This abstract does not necessarily reflect USEPA policy.

**RP152 Effects of Androstenedione on Fathead Minnow (*Pimephales promelas*) Reproduction and Development** Z. DeQuattro, Univ of Wisconsin, Madison, Zoology, Univ of Wisconsin, Zoology; J.C. Hemming, Wisconsin State Laboratory of Hygiene; T. Barry, Univ of Wisconsin. The androgen, 4-androstene 3, 17-dione (A4, androstenedione), was found (>250 ng/L) in runoff from manure fertilized agricultural fields and livestock feeding operations in southern Wisconsin. Androstenedione is an active androgen in fishes and is a substrate in the biosynthetic pathways of other key fish steroid hormones (e.g., testosterone and estradiol-17 $\beta$ ). The purpose of the present investigation was to determine if exposure to exogenous A4, at ecologically relevant concentrations, adversely affects fish reproduction and early development. Reproductively mature fathead minnows were exposed for 26 days to nominal concentrations of 0, 10, 100, and 1000 ng/L A4 in a

flow through system and various key reproductive endpoints (e.g., fecundity, fertilization success) were measured. Fertilized fathead minnow eggs were exposed to nominal concentrations of A4, in static cultures, so to assess early development and hatching success. At termination of the fish short term reproduction assay adults were euthanized, animals were weighed for whole body mass, and secondary sexual characteristics were assessed for abnormalities. Livers were collected to measure Vtg mRNA expression. Gonads were collected and weighed for GSI determination. These data will be discussed along with possible future research directions.

**RP153 Evaluation of the Acute Toxicity of Pesticide Mixtures to *Ceriodaphnia dubia*** J. Westfall, A. DeSmet, S. Turpin, C. Ocab, C. Barton, Sanitation Districts of Los Angeles County. Toxicity thresholds are routinely based on laboratory exposures to single compounds in controlled, laboratory, environments. This scenario is not representative of real world conditions. In this study, we investigated the potential for interactions between multiple stressors on *Ceriodaphnia dubia*. The pyrethroid bifenthrin was coupled with the phenylpyrazole fipronil and the organophosphate chlorpyrifos to evaluate the effect of pesticide blends. Organism response was assessed to each individual stressor, along with two-compound mixtures in 48-hour, static, water-only exposures. Each compound elicited a toxic response, singularly. While the observed effects of the bifenthrin/fipronil combination did not differ substantially from the sum of components' individual effects, the bifenthrin/chlorpyrifos blend appeared to exhibit a synergistic effect. Low chlorpyrifos concentrations paired with non-toxic levels of bifenthrin enhanced the toxic response 200 – 500%. These results suggest that the commonly co-occurring pesticides, bifenthrin and chlorpyrifos are more toxic in tandem than individually.

**RP154 Evaluation of the Effects of POPs in Monitoring Reference Sites in Mexico** M. Guzman Martinez, Universidad Autonoma Metropolitana, Dept of Hidrobiologia; P. Ramirez Romero, Universidad Autonoma Metropolitana, Hidrobiologia, U.A.M. Iztapalapa, Depto. De Hidrobiologia; A.D. Nava Montes, Instituto Nacional de Ecologia, CENICA. Monitoring is an important tool in environmental management; it allows the evaluation of temporal and spatial tendencies of the environmental quality. In order to comply with the Stockholm protocol and other international agreements, a National Monitoring Program of persistent substances (PRONAME, by its Spanish acronym) has been implemented in Mexico since 2007; this includes the long term monitoring of chemicals in different sites with a variety of anthropological influences as well as reference sites. The objective of this work was to develop an ecotoxicological baseline, as a complement to the chemical analysis in two reference sites (Celestun in the Yucatan Peninsula and Manantlan in the State of Jalisco). Sediment and soil samples were obtained and transported to the laboratory. Extraction was performed through sonication and toxicity was evaluated with lettuce seed bioassays. Results for Celestun showed toxicity only in one sample, which was associated to a nearby road that could leach pollutants to the adjacent soil. In a similar fashion none of the Manantlan samples showed toxicity. For this site a second sampling and bioassay analysis confirmed the previous results. When the chemical analysis data of the samples were obtained the lack of toxicity was correlated to the fact that only trace amounts of phenanthrene, anthracene, naphthalene and pyrene were found. To increase the bioassay analysis power another biological assay will be introduced (Microtox) in future samplings, since histological analysis seem to show the beginning of biological problems in Celestun, where more compounds have been detected.

**RP155 Evaluation of Tools Towards Improved Assessment of Copper Bioavailability and Toxicity at Contaminated Sediment Sites** M. Colvin, San Diego State Univ Research Foundation; G. Rosen, SPAWAR Systems Center Pacific, Environmental, SPAWAR Systems Center Pacific, Scientist; I. Rivera-Duarte, SPAWAR Systems Center Pacific, Scientist; P. Earley, B. Swope, SPAWAR Systems Center Pacific. In an effort towards improved understanding of the bioavailability and toxicity of copper (Cu) in marine sediments and, ultimately, an improved ability to accurately assess ecological risk from copper, a series of sediment toxicity experiments were conducted. The experiments employed four different benthic organisms commonly used in sediment quality assessment, with multiple concurrent manipulations and measurements to: a) assess the promising potential for improved prediction of toxicity based on the organic carbon-normalized Cu concentration of the < 63  $\mu$ m sediment fraction, as reported elsewhere; b) support improved predictive models by measurement of physicochemical parameters



in the dissolved and particulate phases; and c) evaluate the utility of passive sampling devices for assessing labile Cu (and Zn) concentrations in sediment porewater. The experimental organisms, two amphipods (*Leptocheirus plumulosus* and *Ampelisca abdita*), one polychaete (*Neanthes arenaceodentata*) and bivalve embryos (*Mytilus galloprovincialis*), were exposed to both whole-sediment copper-spiked sediments and field-collected contaminated sediments using standard protocols. Copper spiked sediments ranged in concentration from 0 to 4500 mg/kg in order to calculate LC50 or EC50 values. All Cu spiking manipulations were conducted under anoxic conditions, allowed to mix for 28 d to achieve equilibrium, and pH adjusted as necessary to prevent unrealistically high concentrations in the porewater. The passive samplers (diffusive gradient in thin films) were coupled with several of the exposures, and compared to both Cu uptake and toxicity. Physiological chemistries measured included pH, SEM/AVS, total and dissolved organic carbon, grain size, and metal concentration in both dissolved and particulate fractions as well as metal concentrations in the polychaete tissues. All species exhibited a clear dose response to the Cu spiked sediments, with isolated responses observed to the field collected sediments. A summary of these experiments and current perspective on the utility of the various tools will be presented.

**RP156 Hypothesis Testing (NOEC) Versus Regression Endpoint Estimation (ECx) in Chronic Ecotoxicity Tests** S.E. Belanger, The Procter & Gamble Company, Environmental Stewardship Organization, The Procter & Gamble Company, Central Product Safety Dept; J. Oris, Miami Univ, Dept of Zoology. A long standing controversy in aquatic toxicology has revolved around the use of hypothesis testing versus endpoint estimation by regression techniques. Hypothesis testing commonly utilizes parametric analysis of variance followed by a post hoc one-sided test to compare treatment means versus the control resulting in NOEC and LOEC conclusions. Endpoint estimation requires the fitting of a regression to the exposure-response curve to estimate a prescribed effect levels such as an EC10. In this study, we summarize representative chronic green algae (*Scenedesmus* and *Pseudokirchneriella*), *Daphnia magna*, and fish toxicity data to quantitatively assess hypothesis versus endpoint estimation techniques as part of a larger investigation devoted to assessing the statistical power of current chronic ecotoxicity tests. The objectives were to evaluate the relationships of NOECs, LOECs, and various ECx levels for each test type and develop objective recommendations based on the empirical results. Algae (OECD 201), *Daphnia magna* (OECD 211), and fish (OECD 210) chronic ecotoxicity test results were derived from complete study reports, including raw data. All NOECs, LOECs, and ECxs were recalculated to harmonize the statistical approaches across studies and to enhance reliability of further comparisons. Comparison of hypothesis test and endpoint estimation included parametric and orthogonal regression. In addition to comparison of effect conclusions within the taxonomic group, an additional analysis was performed using two categories of chemicals 9alcohol ethoxylates and linear alkylbenzene sulfonates) with exceedingly rich chronic toxicity data. In this set of analyses the NOEC or LOEC conclusions were compared to ECx conclusions across a wider array of taxa. Several common themes emerged: (1) The amount of variation explained by the regressions were close to the lines of 1:1 correspondence; (2) slopes of the relationships were all approximately 0.9 to 1.0 and indicate the likelihood of over- or under-predicting is equivalent for both statistical processes; and (3) overall NOEC and EC10 comparisons were consistent with each other indicating that the EC10 does not provide biased conservatism or liberalism for interpreting effects of chemicals. A more complete discussion of advantages and disadvantages of the approaches will be presented.

**RP157 Influence of Select Physiochemical Parameters on the Toxicity of Atrazine to Green Algae** L. Baxter, Univ of Guelph, School for Environmental Science; M. Hanson, Univ of Manitoba, Dept of Environment and Geography, Univ of Manitoba, Faculty of Environment; K. Solomon, Univ of Guelph, School of Environmental Sciences, Univ of Guelph, Centre for Toxicology, School of Environmental Sciences; A. Hosmer, Syngenta Crop Protection, LLC.; R. Brain, Syngenta Crop Protection, LLC., Dept of Environmental Risk Characterization. Surface waters in agricultural regions, particularly in the Midwestern United States, are typified by both spatial and temporal variability of parameters that can influence the growth and development of primary producers. Surface waters in certain areas may experience elevated turbidity and high levels of nutrients, along with low temperatures at the time of spring crop planting. Therefore, applying

toxicity data obtained under only one specific set of conditions can lead to uncertainty in understanding the potential for effects in the field. Existing evidence indicates that increased turbidity, increased nutrients, and lower temperatures can reduce the toxicity of the herbicide atrazine to aquatic plants. Atrazine requires light as a dependent mechanistic co-factor in order to effectively inhibit photosynthesis, growth rate slows under cool temperature conditions, and high nitrogen and phosphorous can up-regulate specific metabolic processes and influence growth characteristics. Consequently, a series of studies evaluating the influence of light intensity (used as a surrogate for turbidity), nutrient content, and temperature on the toxicity of atrazine to the green algae *Pseudokirchneriella subcapitata* were conducted in order to assess the toxicity of atrazine to primary producers under conditions reflective of Midwestern streams in the United States. The results of these algal experiments will be presented.

**RP159 Liver PAH Determination in *Brevoortia tyrannus* and *Brevoortia patronus*** R. Hawke, C.S. Bentivegna, Seton Hall Univ, Biological Sciences; J. Sowa, Seton Hall Univ, Chemistry/Biochemistry. The April 2010 BP® oil spill has caused great concern for ecological impacts in the Gulf of Mexico. Crude oil contains polycyclic aromatic hydrocarbon (PAHs) and oil spills increase the exposure of aquatic organisms to this class of chemicals. The overall health effects of PAHs depends on the time of exposure, the concentration of PAH, and the toxicity of the PAHs in the mixture. *Brevoortia tyrannus* and *Brevoortia patronus* are a filter feeding fish commonly known as menhaden and account for over 40% of the commercial industry fisheries catch. As primary consumers, they are a major link in the food chain. In this study, liver metabolites of PAHs were measured in menhaden using scanning fluorescence spectrophotometry. Standardized curves were effectively established for 3-hydroxypyrene and 2-naphthanol. Liver PAH analyses were performed on fish samples from both Atlantic and Gulf coasts with the anticipation of comparing a BP oil signature with ones from urban environments. Adult menhaden were collected from Delaware Bay, NJ (September- November 2010) the lower Chesapeake Bay, VA (November-December, 2010) and Grand Isle, LA (November 2010). Preliminary analyses revealed that naphthanol-like PAHs were higher in LA fish, while NJ and VA fish had higher levels of hydroxypyrene-like PAHs. Further work needs to be done comparing the fluorescent spectra to other PAH standards.

**RP160 Long-term Variability of Reference Toxicant Tests with Five Freshwater Species** T. Horsley, ENVIRON, Laboratory Services Manager – Ecotoxicology; L. Minella, ENVIRON, Scientist. Reference toxicants are used to evaluate the overall health and sensitivity of toxicity test organisms, and to determine the precision of the tests conducted under specific laboratory conditions. Individual reference toxicant test results are compared to control charts to determine acceptability based on test precision. The coefficient of variation (CV) for the tests (LC50 value for acute tests, IC25 value for chronic tests) provides a measure of test precision. For whole effluent toxicity (WET) testing, EPA compiled frequency distribution data for inter-laboratory CVs for reference toxicant tests from a national database established in 2000. The EPA data allow laboratories to compare their test precision with that of other laboratories. The variability of NaCl reference toxicant tests with five freshwater species used in the National Pollutant Discharge Elimination System (NPDES) discharge monitoring program was measured over a ten-year period. Acute freshwater test species were the cladocerans *Ceriodaphnia dubia*, *Daphnia pulex*, and *D. magna*; and fish species *Pimephales promelas* (fathead minnow) and *Cyprinella leedsii* (bannerfin shiner). The variability associated with chronic *C. dubia* and *P. promelas* tests was also determined. The results of these evaluations were compared to test precision previously documented by USEPA, and the inherent precision associated with each test species and test format (acute and chronic) was also determined.

**RP161 Modeling the Chronic Effects of Pharmaceutical Mixtures on the Life History Strategies of *Ceriodaphnia dubia*: A Multigenerational Study** K. Lamichhane, Univ of North Texas, Environmental Science; T.W. LaPoint, Univ of North Texas; D.L. DeAngelis, Univ of Miami, Dept of Biology. Trace quantities of pharmaceuticals (carbamazepine and ivermectins) are continuously discharged into the environment, causing concern among scientists and regulators regarding their potential long term impact on aquatic ecosystems. These compounds and their metabolites are continuously interacting with organisms at various life cycle stages, and may differentially influence development of embryo, larvae, juvenile, and adult



stages. Rather than choose a single chemical to study, we recognize that aquatic organisms live in a "soup" of chemicals. Hence, to fully understand the cumulative effect of exposure to multiple chemicals, a multigenerational approach will be taken. We will examine and model the chronic toxicity of selected pharmaceutical mixtures at environmentally relevant concentrations on life history and morphological parameters over four generations on *Ceriodaphnia dubia*. Model endpoints will include survival, growth, reproduction, brood size, interbrood duration, sex ratio, and life span. The model will predict the effects of chronic toxicity of *C. dubia* individual behavior under laboratory conditions. Model results will be extrapolated to populations in the natural ecosystem. This approach will establish the consequence of multigenerational exposure to these chemicals and may provide a tool with which to understand the effect of chemical on individual organisms and predict population level effects over time and space.

**RP162 Perfluorinated Chemicals in Surface Waters and Sediments from Northwest Georgia, USA, and Their Bioaccumulation in *Lumbriculus variegatus*** P.J. Lasier, USGS-Patuxent Wildlife Research Ctr., Univ of Georgia; J.W. Washington, USEPA – National Exposure Research Lab; S.M. Hassan, Univ of Georgia; T.M. Jenkins, USEPA – National Exposure Research Lab. Concentrations of perfluorinated chemicals (PFCs) were measured in surface waters and sediments from the Coosa River watershed in northwest Georgia, USA to examine their distribution downstream of a suspected source. Samples from eight sites were analyzed using liquid chromatography-tandem mass spectrometry. Sediments were also utilized in 28-d exposures with the aquatic oligochaete, *Lumbriculus variegatus*, to assess PFC bioaccumulation. Concentrations of PFCs in surface waters and sediments increased significantly below a land-application site (LAS) of municipal/industrial wastewater and were further elevated by unknown sources downstream. Perfluorinated carboxylic acids (PFCAs) with eight or less carbons were the most prominent in surface waters. Those with 10 or more carbons predominated sediment and tissue samples. Perfluorooctane sulfonate (PFOS) was the major homologue in contaminated sediments and tissues. This pattern among sediment PFC concentrations was consistent among sites and reflected homologue concentrations emanating from the LAS. Concentrations of PFCs in oligochaete tissues revealed similar patterns to those observed in the respective sediments. The tendency to bioaccumulate increased with PFCA chain length and the presence of the sulfonate moiety. Biota-sediment accumulation factors indicated that short-chain PFCAs with less than seven carbons may be environmentally benign alternatives in aquatic ecosystems, but sulfonates with four to seven carbons may be as likely to bioaccumulate as PFOS.

**RP163 Quantifying Toxicity Effects of Different Hydrocarbon and Aromatic-based Chemicals via Tissue Extraction Method in *Leptocheirus plumulosus*** G. McMennamy, M-I SWACO, Analytical; K. Nguyen, M-I SWACO, Quality Assurance; P. Tyczynski, M-I SWACO. Continuous efforts have been made by industry over the years to strengthen the sediment toxicity test which uses the benthic organism *Leptocheirus plumulosus*. Different studies have further reinforced the validity of the test which is currently employed by the US Environmental Protection Agency in Gulf of Mexico compliance programs. Research previously carried out involving *L. plumulosus* culture studies has focused on the physiological and biological aspects of the organisms, as well as the primary and secondary modes of ingestion via digestive tract and body cavity areas. The need to further explore the ways in which toxicity effects take place has resulted in study of the toxicity accumulation propensity of the organisms using hydrocarbon and aromatic-based chemicals. The rationale behind the selection of these chemicals comes from its respective worldwide offshore and applicable uses. (These chemicals are known to have different levels of toxicity results from a discriminatory power's standpoint.) Various approaches including radio-isotope, biomarkers and fluorescent tagging by different scientists have been attempted when studying accumulation effects in these amphipods. One of the many challenges has been absence of sufficient sample tissue to assess as these amphipods' sizes are quite small. The purpose of this research is to quantify, by using tissue extraction techniques, the presence of compounds that may affect the health of test subjects by identifying different toxic chemicals and their concentrations via Gas Chromatography-Mass Spectrometry. Furthermore, techniques developed and refined during the ongoing research strengthen the overall efforts in reducing test variability. Preliminary data show both external adhesion and internal presence of the tested chemical via direct ingestion of the amphipods, this by measuring the

presence of hydrocarbon and aromatic-based chemicals in test organisms and comparing this data to the results of the LC50 toxicity data generated during a 10-day benthic sediment toxicity test. Analyses performed on the data suggest a correlation between the presence of test chemicals inside the organisms and that of the LC50 toxicity results. This research will provide invaluable insight into how benthic sediment toxicity testing results and presence of toxic chemicals are correlated.

**RP164 Renovating Fresh Oilfield Produced Waters for Beneficial Uses: Managing Constructed Wetland Treatment Systems for Performance** B. Alley, J. Rodgers, Clemson Univ, Forestry and Natural Resources; J. Castle, Clemson Univ, Environmental Engineering and Earth Sciences. Energy-derived waters (EDWs) are generated in relatively large volumes (~3.3 million m<sup>3</sup> annually), can range from fresh (< 5,000 mg Cl/L) to hypersaline (>40,000 mg Cl/L), and contain constituents that may hinder beneficial use. Fresh oilfield produced water (FOFPW) can contain metals, metalloids, and organics such as oil. Some FOFPWs may be renovated for discharge and irrigation using efficient and effective approaches such as constructed wetland treatment systems (CWTSs). These CWTSs are designed to promote removal pathways resulting in sequestered or non-bioavailable forms of constituents. In CWTSs, pathways and processes for metals (Cd, Cu, Ni, Zn) and oil removal are dependent on specific sediment redox conditions. Sediment redox potential can be controlled by changes in water depth and may affect performance of a CWTS. This experiment was designed to illustrate the role of water depth for controlling sediment redox potential and consequently, performance of a CWTS. Replicate pairs of PS-CWTSs with four sequential cells were designed to provide oxidizing or reducing sediment conditions conducive for removing constituents in simulated FOFPW. The systems were operated with five discrete water depths (15, 23, 33, 46, 56 cm) and a hydraulic retention time of 24 h for each wetland cell. Oxidizing conditions (+15.3 to +250.1 mV) were produced in 15, 23, 33 cm water depths along with greater removal rates for oil (0.01-0.056 day<sup>-1</sup>) than the 46 and 56 cm water depths (-0.001 day<sup>-1</sup>) likely due to differences in rates of aerobic and anaerobic oil degradation. Reducing environments (-65 to -212 mV) were generated in 46 and 56 cm water depths and supported greater removal rates for metals (0.01-0.185 day<sup>-1</sup>) than 15, 23, 33, cm water depths (no change-0.077 day<sup>-1</sup>) which may be attributed to precipitation by sulfides from dissimilatory sulfate reduction. If the sediment redox potential is not within the range conducive for targeted pathways to operate, removal of constituents may not occur regardless of HRT. Water depth can influence sediment redox conditions which may enhance or suppress pathways necessary to transfer or transform constituents to non-bioavailable forms and achieve the desired performance in a CWTS.

**RP165 Sex Determination in AB Strain Zebrafish (*Danio rerio*) Using Anal/Genital Morphometrics** E. Kirichenko, L. White, RUTGERS Univ, Biochemistry and Microbiology; K.R. Cooper, Rutgers, the State Univ of New Jersey, Dept of Biochemistry and Microbiology. The zebrafish (*Danio rerio*) is sexually mature after approximately 90 days post fertilization and can be differentiated by secondary sexual traits. These traits include coloration, body girth and fin shape. Attempts in our laboratory to use these traits for segregating by sex at 1 month were unsuccessful. Our hypothesis was that by examining subtle changes in the anal/genital morphology (AGM) and distance (AGD) from the pectoral fin we could provide earlier indicators of sexual determination. The first study examined sexually mature fish to determine if AGM and AGD were quantifiable and was different between male and female fish. Females had AGD of 0.6 mm or less, while males had an AGD of 1 mm or more. In addition, the difference in the length of the female (> 2.1 mm) and male external gonadal/anal structure (< 2.0 mm) was different. There was no difference in the width of the male or female structure. In a second study fish were individually housed from hatch and observed for AGM and AGD from 4 to 11 weeks. The fish were sedated with MS222 and examined weekly under a stereomicroscope at 40x magnification. At 4 weeks the gonadal pore structure between the male and female could be identified based either on AGM or at later weeks on both AGM and AGD. Histopathology of the organ is being used to confirm the observations made using the stereomicroscope. Currently, this method can be used to determine gender 4 weeks of age and further refinement may allow earlier determinations. (NIEHS ARRA 432298)

**RP166 The Effects of  $\beta$ -Sitosterol Exposure on Fathead Minnow Growth, Survival, and Reproductive Endpoints** C. Flinders, W. Streblow,

R. Philbeck, National Council for Air and Stream Improvement; D.L. Cook, NCASI, Dept of Chemistry; D. Campbell, NCASI; D. Borton, R. Fisher, National Council for Air and Stream Improvement. Phytosterols are a group of steroid alcohols naturally occurring in plants, and therefore a component of pulp and paper mill effluents. Among effluent phytosterols,  $\beta$ -sitosterol is typically found in the greatest concentration. Studies examining the effects of  $\beta$ -sitosterol on aquatic organisms are limited, but estrogenicity; and effects on egg size and survival, juvenile and adult survival have been reported at concentrations of  $\beta$ -sitosterol that were  $< 20 \mu\text{g/L}$ . We exposed fathead minnow eggs to four  $\beta$ -sitosterol concentrations of 20, 65, 218, and 689  $\mu\text{g/L}$  based on measurements of samples from exposure aquaria, a dilution water only (negative) control, an acetone control (0.6 ml/L acetone,  $\beta$ -sitosterol carrier), and ethinylestradiol (EE2, 15 ng/L, positive control). Fish were monitored through larval, juvenile, and adult stages, with adult fish allowed to spawn to determine egg production and hatchability. Calculated endpoints included survival; weight; gonadosomatic (GSI) and hepatosomatic index (LSI); condition factor; egg size, production, and hatchability; numbers of tubercles on heads of males and females; gonad histology; and steroid hormone (estradiol, testosterone, 11-ketotestosterone) and vitellogenin concentrations. Findings from this study differed from previous life cycle studies. There was no significant difference in fish growth or mortality with  $\beta$ -sitosterol exposure. Although there was a decrease in egg production in treatment groups relative to the negative control, there were no significant treatment differences relative to the acetone control suggesting that reductions were due to acetone and not  $\beta$ -sitosterol. All fish exposed to EE2 showed female characteristics but did not spawn. Histopathological examination of gonadal tissue showed no significant differences in the reproductive development or spawning capability of male or female fathead minnow with  $\beta$ -sitosterol exposure. Male fish exposed to EE2 were found to have ovarian tissue, and none were spawning capable. Hormone and vitellogenin data are still being evaluated. Preliminary findings from this study indicate that  $\beta$ -sitosterol exposure has a minimal impact on fathead minnow survival, growth, and reproduction, and suggests that fish response to  $\beta$ -sitosterol exposure may be species-specific or that methodology differences from earlier studies contributed to differences in fish response.

**RP167 Toxicity of Chemically Dispersed Crude Oil to Herring Embryos** C. Greer, Queen's Univ, Biology; P.V. Hodson, Queen's Univ, School of Environmental Studies. Significant declines in Pacific herring (*Clupea pallasii*) populations have followed two major oil spill events in the United States, the Exxon Valdez oil spill and the Cosco Busan oil spill. As a result of oil-related declines, the literature has been dominated by studies that have assessed the risk to Pacific herring. Although oil tanker routes also coincide with Atlantic herring (*Clupea harengus*) spawning grounds, little research has been done to study the risk of oil exposure to Atlantic herring. The main objectives of this research were to determine if chemical dispersion of crude oil increases the toxicity to herring embryos and if Atlantic and Pacific herring embryos respond similarly to oil exposure. If the two species are sufficiently similar, available knowledge on Pacific herring can be expanded to include Atlantic herring for risk assessment. Chemical dispersion increased the concentration of oil in the water column by 100-fold, resulting in a significant increase in toxicity. Additionally, Atlantic herring appeared to be four times more sensitive to chemically dispersed oil than Pacific herring embryos, as determined by the percentage of normal embryos at hatch and the incidence of blue sac disease. This indicates that chemical dispersion of oil increases the risk to herring embryos and that exposure limits for risk assessment should be set conservatively to protect both species.

**RP168 Transgenerational Effects of Contaminants: Does Parental Cadmium Exposure Affect the Cadmium Tolerance of Offspring?** S. Plautz, C. Salice, Texas Tech Univ, The Institute of Environmental and Human Health. Maternal effects are non-genetic influences on offspring phenotype that occur via the phenotype of the mother or the environment she experiences. Maternal effects are one of the most important determinants of offspring phenotype and are thus influential in the adaptation and evolution of populations. When mothers experience changes in environmental conditions, they may produce offspring that are more tolerant of the new conditions, thus adaptively modifying their offspring. However, in other cases mothers may produce offspring with a lower tolerance or a lower general fitness. This effect of the maternal environment on offspring phenotype has thus far received little attention in toxicology, but the available data demonstrate an effect of maternal contaminant exposure on offspring contaminant

tolerance and fitness. This is important because maternal influences on contaminant tolerance in offspring, whether in the direction of increased or decreased tolerance or fitness, will have a significant impact on population adaptation to contaminants and ultimately population persistence. To further explore the effects of maternal contaminant exposure on offspring, we exposed a laboratory strain of *Biomphalaria glabrata* snails to 0, 2, or 10  $\mu\text{g/L}$  cadmium for 7 days, after which they were transferred to clean water. We then collected egg masses and exposed them to 0 or 2  $\mu\text{g/L}$  cadmium in a factorial design for the duration of egg development. When hatchlings were 12 days old, we exposed them to 200  $\mu\text{g/L}$  cadmium and recorded time to death with observations every 8 hours for 3 days. Among juveniles with cadmium-exposed parents, preliminary data indicate a marginally significant difference in the effect of the parental cadmium concentration ( $p=0.091$ ), with snails whose parents were exposed to 10  $\mu\text{g/L}$  cadmium displaying lower cadmium tolerance. Preliminary data also indicate an interactive effect of parental cadmium exposure and egg mass cadmium exposure on juvenile survival in cadmium over time ( $p=0.028$ ). Taken as a whole, these results suggest that cadmium exposed parents do not impart enhanced cadmium tolerance to offspring in *B. glabrata*. We are currently exploring similar questions regarding the transgenerational effects of cadmium using wild caught *Physa pomilia* snails. Our results and results from similar studies on transgenerational effects of contaminants have important implications for understanding how populations respond to anthropogenic stressors.

**RP169 A Proteomic Approach to Biomarker Discovery in *Heterocypris incongruens* Exposed to Heavy Metals Using SELDI-TOF MS** Y. Kim, J. Son, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences; H. Mo, Korea Univ, Division of Environmental Science and Ecological Engineering; Y. Lee, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, Environmental Science and Ecological Engineering; M. Kim, Korea Univ; K. Shin, Hankuk Univ of Foreign Studies; K. Cho, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, # 407, Division of Environmental. The use of SELDI-TOF (Surface enhanced laser desorption ionization-time of flight) technique has been increased recognition as a tool for screening and discovering biomarkers in environmental monitoring. In this study, the changes in protein peaks in *Heterocypris incongruens* were compared after six day exposure to the sublethal concentrations of three different heavy metals, cadmium, copper and lead using Q10 and CM10 protein chips. Obvious differences in the peak patterns (up- and down- expression of protein in *H. incongruens*) between the heavy metals were observed, implying that the effects of three heavy metals to protein expression are different. Construction of the decision tree classification algorithm was performed on the significantly different protein peaks. The decision tree algorithm applied in SELDI-TOF MS was tested for the validation group. The results showed that the sensitivity and specificity in the validation group were very high. This proteomics approach can be considered to be a valuable tool for the development of biomarker.

**RP170 A Quantitative Food Web Approach for Estimating Selenium Flux in the Colorado River in Grand Canyon** D. Walters, USGS, Fort Collins Science Center, USGS, Ecologist; E. Rosi-Marshall, Cary Institute for Ecosystem Studies; W. Cross, Montana State Univ, Ecology; T. Kennedy, USGS, Grand Canyon Monitoring and Research Center; C. Baxter, Idaho State Univ, Biological Sciences. Selenium (Se) biomagnifies in aquatic food webs and is toxic to fishes and terrestrial piscivores. Se concentrations exceed fish and wildlife toxicity thresholds throughout the Colorado River Basin. The Colorado River within Grand Canyon experiences enormous Se loads (30 metric tons/y of dissolved Se), yet Se data are lacking for this part of the river due to its remote access. We sampled the Colorado River food web (organic matter, invertebrates, fishes) for Se at six sites spanning ~360 river kilometers downstream of Glen Canyon Dam. Our objectives were to quantify the magnitude of Se contamination in this reach and to estimate Se fluxes within the food web. We used quantitative food web methods, which combine data on animal secondary production, gut contents analysis, energetic efficiencies, and Se concentration data, to estimate Se flux within the food web. Mean Se concentrations for organic matter, macroinvertebrates, and fishes were 1966, 3752, and 2049 ng/g wet weight, respectively, well above established risk thresholds. We estimated total Se flux of 825  $\mu\text{g}/\text{m}^2/\text{y}$  through the food web at our upstream-most site (the Glen Canyon

reach). The main pathways of Se flux were from diatoms and amorphous detritus to the benthic invertebrates *Gammarus* and New Zealand mudsnail. Flux to rainbow trout (the dominant fish species) was  $4 \mu\text{g}/\text{m}^2/\text{y}$ , and was primarily from amorphous detritus and algae. Se flux through the food web is approximately  $2.7 \text{ kg}/\text{y}$  within the 25 km Glen Canyon reach, a miniscule proportion of the annual Se load. Even though Se uptake within the food web is small relative to loads, concentrations within organisms reflect high risk to Se exposure.

**RP171 A Survey of Pharmaceuticals and Personal Care Products (PPCPs) in a Binational River and Their Potential Impacts on Basal Trophic Levels** D.A. Martinez Gomez, Univ of Texas at El Paso, Environmental Science Program; S. Baca, Univ of Texas at El Paso; R. Guerrero, Univ of Texas at El Paso, Dept of Chemistry; E. Walsh, Univ of Texas at El Paso, Dept of Biological Sciences. The Rio Grande is an important binational aquatic system serving as a primary source of drinking, irrigation and recreational water. As in other regions worldwide, the river receives wastewater inputs from industrial, urban and agricultural uses. Wastewater treatment plants are not designed to remove the pharmaceuticals and personal care products (PPCPs) pollutants and thus effluent inputs into surface waters are of increasing concern. Concentrations of PPCPs in the El Paso/Ciudad Juarez stretch of the Rio Grande have not been determined nor have their potential impacts on aquatic life. A survey of 14 selected PPCPs is being conducted in four sites along this stretch. Concentrations of PPCPs are being analyzed by HPLC-MS/MS following EPA Method 1694. The rotifer *Platynus patulus*, a basal member of riverine food webs, is being used to test for acute and chronic toxicity of 4 selected PPCPs (caffeine, acetamidophenol, cotinine, sulfamethazine). One population from the Rio Grande (downstream of the El Paso/Ciudad Juarez metroplex) and other from a remote site near Big Bend National Park (BBNP, TX) are being tested in order to compare populational differences in sensitivity. Preliminary results show the presence of caffeine, trimethoprim, erythromycin, cotinine and sulfamethazine in the river. In toxicity tests, *P. patulus* showed higher sensitivity to caffeine (BBNP: 48 hr  $\text{LC}_{50}$  425 mg/L; El Paso/Ciudad Juarez: 372 mg/L) as compared to *Daphnia magna*. For the BBNP population, the acetamidophenol  $\text{LC}_{50}$  (25 mg/L) is also lower than that for *D. magna* ( $\text{LC}_{50}$  40.8 mg/L); thus *P. patulus* may be a more sensitive bioindicator of toxicity of these PPCPs. For the BBNP population, chronic toxicity was assessed using a modified life cycle test in which the population growth rate ( $r$ ) was calculated over a range of concentrations (caffeine and acetamidophenol). For caffeine exposures, a significant decline in population growth occurred as concentrations increased. After 5 days there was a significant decrease in population growth rate between the control ( $0.23 \pm 0.02$ ), 100 ppm ( $0.07 \pm 0.01$ ), 200 ppm ( $0.06 \pm 0.012$ ), and 300 ppm (one replicate remained at day 6;  $-0.08$ ). Increasing acetamidophenol concentrations resulted in the production of non-viable eggs and, thus, negative population growth rates. Tests with additional compounds are underway. Results from this study will be provided to resource managers in order to help them better protect our water supplies.

**RP172 Acute and Chronic Toxicity of Cobalt to the Planarian *Dugesia dorotocephala*** A. Sternberger, Colorado State Univ, Center for Environmental Medicine, Colorado State Univ; O.R. Arnold, Colorado State Univ, Center for Environmental Medicine, Colorado State Univ, Environmental and Radiological Sciences; H.S. Ramsdell, Colorado State Univ, Center for Environmental Medicine. *Dugesia dorotocephala* is a useful invertebrate species for aquatic toxicity testing. This free-living flatworm is easy to culture and their size facilitates convenient observation and easy handling. These studies were intended to define the acute lethal concentration of a soluble cobalt salt ( $\text{CoCl}_2$ ) for this species, to identify a sensitive sublethal effect for chronic toxicity tests and to understand how hardness may influence cobalt toxicity. Planaria were exposed to cobalt in reconstituted water formulated to achieve the required hardness value while accounting for the contribution of  $\text{Co}^{+2}$ . Exposures were conducted using static renewal protocols. In moderately hard water, a 96 hour  $\text{LC}_{50}$  of 27 mg Co/L was observed. After 168 hours of exposure, an  $\text{LC}_{50}$  of 12 mg Co/L was found. The  $\text{LC}_{50}$  values increased progressively with water hardness. A 60-day exposure to  $\text{Co}^{+2}$  was conducted in moderately hard reconstituted water. Fissioning, the mode of reproduction for this asexual planarian, was significantly suppressed at the lowest Co concentration tested, 1 mg/L. Regeneration rates and behavioral responses were less sensitive to  $\text{Co}^{+2}$  exposure. In tests where hardness was held constant but varying ratios of  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  concentrations were employed, it was found that  $\text{Ca}^{+2}$  exerted a greater protective effect against

acute  $\text{Co}^{+2}$  toxicity than  $\text{Mg}^{+2}$ . Further studies are in progress to define a chronic no-effect level for  $\text{Co}^{+2}$  in *D. dorotocephala*.

**RP173 Acute Copper Toxicity and Acclimation Using Behavioral Endpoints of Shoaling and Predator Avoidance in the Least Killifish (*Heterandria formosa*)** S. Vogt, Univ of Louisiana at Lafayette, Biology; P.L. Klerks, Univ of Louisiana at Lafayette, Dept of Biology; A. Billock, Univ of Louisiana at Lafayette, Biology. Many toxicity studies use mortality as an endpoint to determine a response to heavy metals. Due to copper's neurotoxic effects, we were interested in whether a behavioral response, shoaling or predator avoidance, could be used as an indicator of copper toxicity and acclimation. Shoaling is defined as a social assembly of fish that swim with little to no structure. To answer the question of acute toxicity, we exposed least killifish (*Heterandria formosa*) to 0, 25, 50, or 100  $\mu\text{g}/\text{L}$  Cu for 2 hours. For copper acclimation, fish were pre-exposed for 7 days to either 0 or 15  $\mu\text{g}/\text{L}$  Cu and then exposed for 2 hours to 50  $\mu\text{g}/\text{L}$  Cu. Fish were put in an observation aquarium that contained a clear plexiglass divider that contained 15 shoaling least killifish or 3 predators. The other side of the divider allowed a test fish to be monitored by video for 3 minutes. Videos were analyzed for different parameters of shoaling behavior: time to first shoaling event, total shoaling time and time spent near surface. Videos were also analyzed for different parameters of predator avoidance: time spent near bottom, time spent at surface, total time away from predator. Preliminary experiments have shown a significant decrease in total shoaling time at exposure to copper compared to controls ( $F_{3,58}=5.1137$ ,  $p=0.0033$ ). Ongoing research will examine the presence of copper acclimation using shoaling and predator avoidance.

**RP174 Acute Toxicity of Insecticides on the Calanoid Copepods, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, of the San Francisco Estuary** S.A. Lesmeister, Univ of California at Davis, Ecology, Veterinary Medicine: Anatomy, Physiology and Cellular Biology. The calanoid copepods, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, are a critical link between primary producers and fish in the San Francisco Estuary (SFE). Since these meso-zooplankton play an important role as food sources to larval fish and pelagic organisms, factors affecting their changes in abundance in recent years warrants an investigation. One potential source of decline is insecticide exposure from nearby agricultural and urban run-off into the estuary. The goal of this study was to determine and contrast the acute effects of insecticides, Chlorpyrifos- an organophosphate insecticide, Permethrin and Bifenthrin-pyrethroid insecticides, to *E. affinis* and *P. forbesi*. The 96-hour median lethal concentration ( $\text{LC}_{50}$ ) testing indicates that *E. affinis* (803.2 ng/L) is more sensitive to Chlorpyrifos than *P. forbesi* (1211.9 ng/L). However, *P. forbesi* is two-times more sensitive than *E. affinis* to Permethrin ( $\text{LC}_{50}$  values 86.0 and 158.1 ng/L, respectively). For Bifenthrin, *E. affinis* showed an  $\text{LC}_{50}$  value of 13.3 ng/L, while values for *P. forbesi* are 19.40 ng/L. Ongoing studies are investigating the  $\text{LC}_{50}$ s of five more commonly used pesticides in the SFE, including Malathion, Chlorothalonil, Fipronil, and Diuron. Results from these studies are anticipated prior to the conference. Results indicate species specific differences in response to insecticides. Shifts in the types and timing of insecticide use may cause a decline in copepods abundance, and ultimately have a negative impact on the SFE food web.

**RP175 Alterations of Biochemical Indicators in Hepatopancreas of the Apple Snail, *Pomacea canaliculata*, from Paddy Fields in Taiwan** T. Hsieh, Y. Chiu, Kaohsiung Medical Univ, Biomedical Science and Environmental Biology; D. Huang, Chia Nan Univ of Pharmacy and Science, Environmental Resources and Management; C. Chen, Chia Nan Univ of Pharmacy and Science, Environmental Resources and Management, Chia Nan Univ, of Pharmacy and Science. Although organic farming becomes popular, most of agriculture activities in Taiwan are still based on traditional methods which rely heavily on pesticides. The ecological impacts for application of pesticides on paddy fields are usually not fully acknowledged. Introducing alien species into our environment also confound the problems, especially for apple snail, *Pomacea canaliculata*, which become prevalent and a major ecological disaster in Taiwan. The purpose of this study is to investigate the status of apple snails collected from paddy fields, with or without pesticide application, throughout the islands using different biochemical indicators. Apple snails were sampled from Hsinchu, Yunlin, Tainan and Pingtung areas in Taiwan. Several enzymatic activities, namely monooxygenase (MO), glutathione-S-transferase (GST), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as concentrations of urea



nitrogen, creatinine and vitellogenin (Vtg) in hepatopancreas of the animals were measured and compared with those from the controls raised in our laboratory. Our result shows that the activities of MO, GST, ALT and AST in snails from some of the paddy fields were higher than those in the controls. The highest were observed in snails from a paddy field using pesticides heavily in Pingtung. By comparing to controls, the differences of MO, GST, ALT and AST were up to 8, 13, 3, 5 folds, respectively. For VTG, creatinine and urea nitrogen, levels in snails from the paddy field were also found to be significantly higher than those in the controls, as well as others fields. Snails from other fields using pesticides also shows altered either enzymatic activities or levels of VTG, creatinine and urea nitrogen, although some of the differences varied and may not be consistent. We are still gathering more data and further results will be summarized and presented at the conference.

**RP176 An Ecotoxicoproteomic Approach (SELDI-TOF MS) to Biomarker Discovery in Eggs of *Ephemera orientalis* McLachlan Exposed to Heavy Metals** H. Mo, Korea Univ, Division of Environmental Science and Ecological Engineering; S. Lee, Nanotextech Inc.; K. Cho, Korea Univ, Division of Environmental Science and Ecological Engineering, College of Life and Environmental Sciences, Korea Univ, # 407, Division of Environmental. Proteomics provide potential in the discovery of new sensitive biomarkers for environmental pollution. We utilized SELDI-TOF MS (surface enhanced laser desorption/ionization time-of-flight mass spectrometry) to analyze the proteomic profile of mayfly eggs (*Ephemera orientalis*) exposed several heavy metals (lead, mercury, copper, cadmium and chromium). While eggs of *E. orientalis* were very sensitive to lead, mercury and copper, but high concentration of cadmium and chromium did not affect on egg hatching. The protein peak patterns of *E. orientalis* eggs exposed to heavy metals were analyzed and compared using ProteinChip® Data Manager software (version 3.0, Bio-Rad, USA). This work could be the basics to develop the novel biomarker for aquatic ecological monitoring.

**RP177 An Evaluation of the Incidence of Testicular Oocytes (Intersex?) in Largemouth Bass as a Function of Land-use and Season** T. Gross, Environmental Resource Consulting, Univ of Florida. Several reports have suggested the presence of testicular oocytes (female germ cells) in fish as an indicator of intersex and exposure to endocrine disruptors. However, these studies have also reported a significant incidence of testicular oocytes in animals from reference and exposed sites. The baseline incidence of testicular oocytes in is not well documented nor clear that this is an intersex condition related to endocrine disruptors. The current study utilized both: a historic dataset with sites across the US and measures of land-use conducted during the 1990's and analyses of largemouth bass within the lower St Johns River basin in FL. The historic dataset had largemouth bass and carp from sites across the US with land-use designations: agriculture, urban/industrial, and reference. A representative portion of gonad was used for histopathology to assess repro stage and incidence of testicular oocytes. The FL study utilized bass from a reference site to characterize the incidence of testicular oocytes in male largemouth bass with season and year. The incidence in the historic dataset ranged from 0% to 44% of fish collected per site for bass, and from 0% to 12% per site for carp and the incidence did not differ with land use. For largemouth bass in the St Johns River the incidence of oocytes across the total gonad using serial sections rather than a representative portion was assessed. Analyses indicate that the incidence is highest near both apices of each gonad and not consistent across the total gonad and the incidence of testicular oocytes ranged from 38 to 46% across seasons, years and sites. These data suggest that the previous utilization of a representative portion may not have been adequate for the assessment of incidence. These data suggest that appropriate and validated techniques are critical to the accurate assessment of the incidence of testicular oocytes in fish and that testicular oocytes may be a normal remnant of sexual differentiation that varies with fish species. The relationship to endocrine disruptors and/or contaminants is not clear but exposure would have to occur during early development rather than in adults. Testicular oocytes were always at early germ cell stages regardless of season, site, land-use or year. Future efforts should quantify the number of testicular oocytes as well as incidence and utilize controlled contaminant exposures.

**RP178 An Evaluation of the Sensitivity of Freshwater Mussels in 7-day Effluent Toxicity Tests Compared to Commonly Tested Species** J.L. Kunz, USGS; C.G. Ingersoll, USGS, Columbia Environmental Research Center; N. Wang, US Geological Survey, Columbia Environmental Research

Center; T. Augspurger, US Fish and Wildlife Service, Ecological Services; C. Hollenkamp, NCDWQ, ESS ATU. The federally endangered Tar River spiny mussel (*Elliptio steinstansana*) is endemic only to the Tar River and Neuse River systems in North Carolina. The primary threats to the species and its habitat appear to be stream impacts associated with conversion of forest lands and loss of forested riparian buffers, impoundment and water withdrawal, and silt and other pollutants from forestry, agriculture, and urbanization. The primary objectives of this study were to (1) evaluate the sensitivity of federally endangered Tar River spiny mussel and two non-endangered surrogate mussels (yellow lance, *Elliptio lanceolata* and notched rainbow, *Villosa constricta*) to currently permitted effluents and reference toxicants (copper, ammonia, and synthetic effluent), and (2) compare the mussels' sensitivity to that of two commonly tested species (cladoceran, *Ceriodaphnia dubia* and fathead minnows, *Pimephales promelas*). Short-term (7-d) static-renewal tests were conducted with yellow lance, notched rainbow, the cladoceran, and fathead minnow in two permitted effluents collected in North Carolina. Preliminary results indicate that the two mussel species were equally or more sensitive to the two effluent samples compared to the cladoceran and fathead minnow. Additional effluent tests and the reference toxicant tests with mussels and the two commonly tested species are ongoing. Tar River spiny mussels have been successfully captively propagated through this project; their sensitivity relative to the surrogate mussels will be assessed when propagated in sufficient numbers for toxicity testing.

**RP179 Are Male Bluegills (*Lepomis macrochirus*) Experiencing Altered Reproductive Output Due to Exposure to Chlorinated Hydrocarbons?** J. Hillis, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center; M. Lydy, J. Garvey, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology. For the past two decades, the chemical disruption of the endocrine system by xenobiotics has been a topic of discussion and concern. There are many indicators showing that endocrine disrupting chemicals (EDCs) in wild fish could potentially disrupt reproduction. This includes elevated vitellogenin levels in males, high incidence of intersex, and altered steroid profiles. Some chlorinated hydrocarbons (CHs) such as polychlorinated biphenyls and organochlorine pesticides act as EDCs and have been shown to be estrogenic or anti-androgenic. Chlorinated hydrocarbons continue to persist in the environment despite being restricted from use in the United States for three decades. This includes reservoirs where fishing is an important source of recreation. Bluegills (*Lepomis macrochirus*, BLG) play an important role in these food webs. Bluegills have been stocked extensively in impoundments as a forage fish for largemouth bass (*Micropterus salmoides*) and as sport fish. Building off this knowledge, the overall objective of this study was to quantify the concentration of CHs in male bluegill in Illinois and relate these concentrations to the incidence of gonad abnormalities and the gonadosomatic index. Although there is much evidence suggesting that CHs cause adverse reproductive effects, there still is little evidence to suggest that EDCs are influencing fish and other organisms at the population level. The proposed research addresses this question by conducting a reproductive trial using wild-caught fish from lakes with whole-body CH concentrations and a control lake with less than background CH concentrations. Preliminary work has detected organochlorine pesticide (DDT, DDD, DDE, and  $\alpha$ -chlordane) residues ranging from 3 to 65 ng/g (dry weight) in whole body tissue samples.

**RP181 Assessment of Chronic Toxicity of Upper Columbia River Sediments to Early Life Stage White Sturgeon** D.W. Vardy, Univ of Saskatchewan, Toxicology Centre, Univ of Saskatchewan, Ph.D candidate; J.A. Doering, S.C. Beitel, B.J. Tendler, Univ of Saskatchewan; R. Santore, A.C. Ryan, HDR|HydroQual; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; M. Hecker, Univ of Saskatchewan, Toxicology Centre. Sturgeon (Acipenseridae) populations are threatened throughout the world and have been decreasing over the past century in North America, Asia, and Northern Europe. In North America, populations of white sturgeon (WS; *Acipenser transmontanus*) are declining in the north-western USA and British Columbia, Canada, primarily due to poor annual recruitment. Pollution has been hypothesized as one potential cause for poor recruitment in the Columbia River (CR) between Grand Coulee Dam in the USA, and the Hugh L. Keenleyside Dam in southern British Columbia, Canada. Potential past and/or present pollution sources include metallurgical facilities, pulp and paper

mills, and other industrial and municipal sources. Given the epibenthic nature of WS, there is the potential for substantial exposure to contaminated sediments within the CR. Specifically, there are concerns about the potential toxicity to WS early life stages (including the early hiding stage where fry are in proximity to sediments) of contaminants such as metals that are associated with sediments. The present study evaluated the effects of metals in sediments collected from the CR on early life stage WS. WS were exposed to field-collected sediments from areas considered to be suitable WS habitat, and containing a range of metal concentrations. Reference sediments were collected from sites in Canada, upriver from the study area. Studies were conducted at the Aquatic Toxicology Research Facility, Univ of Saskatchewan, Saskatoon, SK, under simulated fluvial, flow-through, conditions from hatch to 60 days post hatch. Survival in the references was greater than 75% and preliminary data evaluation revealed no obvious trends in survival or growth among groups exposed to CR sediments.

**RP182 Benthic Invertebrate Exposure and Chronic Toxicity Analysis for cVMS Materials – A Probabilistic Approach and Comparison to the Target Lipid Model** K.B. Woodburn, Dow Corning Corporation, Health & Environmental Sciences; D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101); A. Redman, P. Paquin, Hydroqual. Lipophilic chemicals, such as the cyclic volatile methylsiloxane materials octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), adsorb extensively to particles and surfaces in aqueous systems, making sediments a key sink when performing risk assessment evaluations. A widely accepted step for estimating the possible risk posed by such chemicals to sediment-dwelling species is to compare the observed sediment concentration with either published ecotoxicity guidelines or to chronic no-observed effect concentrations (NOECs) from toxicity testing with benthic invertebrates. The comparison of field concentrations with chronic NOEC levels can be done with simple worst-case simulations or using a probabilistic distribution approach. In this work, probabilistic methods were used to compare residues of D4 and D5 from sediments and organisms collected in Canada, the United States (Lake Pepin, Minnesota), the UK, and Nordic countries, including Inner Oslofjord to chronic NOEC values determined using EPA/OECD test species such as *Chironomus tentans*, *Chironomus riparius*, *Hyalella azteca*, and *Lumbriculus variegatus*. The risk assessment was extended using ecotoxicity data on more than 90 chemicals and 50 species using the Target Lipid Model (TLM) database. Comparisons were made using sediment levels on a dry weight and organic carbon basis, and with organism residues on a lipid-adjusted basis. Probabilistic endpoints of 95% exposure and 5% chronic NOEC were extrapolated from the data, which were fit using log-normal assumed distributions. The cVMS acute-to-chronic ratios (ACRs) were consistent with TLM data and with a narcosis mode of action (average ACR of 2-5). Using either probabilistic techniques or the TLM database, field D4 and D5 concentrations were far below chronic threshold NOEC values with benthic invertebrates, therefore very limited risk appears to exist for benthic invertebrate species with these materials.

**RP183 Benthic Macroinvertebrate Metrics Correlated with Heptageniid Mayfly Whole Body Metal Concentrations in a Stream Near a Uranium Enrichment Plant** A. Wigginton, Univ of Kentucky, Dept of Civil Engineering, Univ of Kentucky, Dept of Biology; R. Umbstead, Univ of Kentucky, Civil Engineering; J. Walker, Univ of Kentucky; B.F. Brammell, Asbury Univ, Natural Sciences Dept. The Paducah Gaseous Diffusion Plant (PGDP) is currently the only active uranium enrichment facility in the United States. It is located in McCracken County, Kentucky and is bordered to the west by a first order stream, Bayou Creek (BC). The plant generates several effluents that contain output from various industrial processes including sewage waste; roof, surface, and parking lot drainage; settling pond effluents; and air conditioning condensate. Sites along Bayou Creek were sampled for aquatic macroinvertebrates using a Surber sampler and steel scoop on October 24-25, 2009. At the same time, Heptageniid mayflies were sampled and analyzed for metal contamination using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). When considering macroinvertebrate populations in general and Chironomid populations in particular, several metrics, including the Hilsenhoff Biotic Index, Shannon Weaver and Simpson Diversity Indices, the Community Loss Index, and the EPT/Chironomid Index indicated a reduction in water quality near the plant compared to upstream reference sites. Heptageniid mayflies collected from areas of BC adjacent to or downstream from the PGDP showed

significantly different average metal concentrations for Cd, Co, Cu, Mn, Ti, Zn (ANOVA, Tukey HSD,  $p < 0.05$ ), and also, at a lower level of confidence, Ba, Fe, and P (ANOVA, Tukey HSD, 0.05)

**RP184 Benzotriazole UV Stabilizers and Polycyclic Musks in Bivalves from Japanese Coastal Waters: Occurrence and Source Determination** A. Nishidome, H. Nakata, R. Shinohara, N. Shikata, Kumamoto Univ. Recently, the occurrence and contamination of persistent personal care products, such as benzotriazole UV stabilizers (BUVSs) and polycyclic musks have been reported in the environment. The detection of these compounds in marine mammals and seabirds suggests their bioaccumulation potentials through the aquatic food-chains. BUVSs and musks were detected in mussels collected from Asian countries, indicating their widespread contamination in Asian coastal regions. However, detail information is lacking on the concentrations and distribution of BUVSs and synthetic musks in Japanese coastal waters. In this study, we analyzed four BUVSs and two polycyclic musks in bivalves collected from 57 stations of Japanese coastal waters. BUVSs, UV-326, UV-327, and UV-328 were detected in all samples analyzed, at the mean concentrations of 1,230, 150, and 336 ng/g (lipid wt.), respectively. A significant correlation between UV-327 and UV-328 were found in bivalves, which suggest that these compounds might have been originated from same sources. To determine the source of BUVSs and musks in the environment, influent, effluent, and sludge samples of WWTPs and road dusts were analyzed in this study. BUVSs were detected in all WWTP samples, and UV-326 was the dominant compounds in influents (mean: 46 ng/L), followed by UV-328 (34 ng/L). High concentrations of HHCB and AHTN were found in liquid and solid samples of WWTP, suggesting the present use of BUVSs and musks in cosmetics and household materials. BUVSs and musks were also detected in road dusts. Significant correlations were obtained between BUVSs concentrations and traffic densities. These results indicate that WWTPs and road dust may be a potential source of BUVSs and musks in the aquatic ecosystems.

**RP185 Best Management Practices as Tools for Nutrient Reductions in the Lower Mississippi Alluvial Valley: What Do We Really Know** R. Kroger, Mississippi State Univ, Dept of Wildlife, Fisheries and Aquaculture, Mississippi State Univ; A. Sharpley, Univ of Arkansas, Crop, Soil and Environmental Sciences. Agricultural best management practices are utilized to reduce and mitigate nutrient concentrations and loads leaving landscapes, thus reducing the toxicological and ecological impacts on downstream aquatic systems. The current study provided a review of the scientific literature to create nutrient reduction efficiency percentages for BMPs in row-crop agriculture within the Lower Mississippi Alluvial Valley (LMAV). The review consisting of multiple agricultural and biological database searches yielded 75 articles that initially were included (search terms: BMP, agriculture, nutrients, Mississippi). Utilizing specific criteria of row-crop agriculture, clay/silt loam soils; slopes 0-5%, and LMAV resulted in 16 studies across multiple BMPs for inclusion. Best management practices included tillage management, wetlands, winter rice field management, subsurface drainage and vegetated drainage ditches. Total P and total nitrogen as well as nutrient species percentage reduction information was parsed from the scientific studies. The lack of replicate studies for BMPs within the LMAV limits the ability of discerning nutrient specific reduction efficiencies associated with various BMP applied to the agricultural landscape. This study was limited in its search criteria to row-crop agriculture in the LMAV, and ignored potentially relevant BMP studies from elsewhere in the country. This was decided extremely important as site specific conditions dictate the reduction efficiency of applied BMPs and as such data from elsewhere would be neither transferable nor applicable. This study has highlighted the lack of scientifically validated reduction efficiency information on BMPs and suggests increased BMP validation to improve understanding, which would enhance BMP driven models for landscape stewardship.

**RP186 Bioavailability and Toxicity of Sediment-associated Uranium to the Freshwater Midge *Chironomus dilutus*** S.E. Crawford, K. Liber, Univ of Saskatchewan, Toxicology Centre. The anticipated increase in demand for nuclear power production will result in the increased mining of uranium (U), which raises the potential for increased U levels in adjacent aquatic ecosystems. Interactions between dissolved U and solid phases can result in sediment U concentrations that are several orders of magnitude higher than those in the water column. Despite high concentrations of U found downstream from some older Canadian U mining operations, little is known



about the bioavailability of U from solid phases with respect to effects on freshwater benthic invertebrates. Physicochemical characteristics of sediment have been found to influence the bioavailability and toxicity of U and other trace metals associated with sediments. Thus, the purpose of this research is to further investigate the influence of key sediment characteristics on the bioavailability and toxicity of sediment-associated U using the freshwater midge *Chironomus dilutus* as a model organism. The objective of our first study is to determine if different clay minerals with different surface chemistries differentially influence the bioavailability of sediment-associated U in a series of 10-d sediment toxicity tests. The influence of different U sorption capacities on U bioavailability will be further investigated with experiments on particle size distribution, which may also play an important role in the bioavailability of U. The growth, survival and bioaccumulation of U in *C. dilutus* are examined and compared among treatments and experiments. All water (overlying and pore water), sediment and tissue samples are analysed for U using inductively coupled plasma-mass spectrometry (ICP-MS). The different sorption properties of different sediment components may prove to be important in the risk assessment of U contaminated sites, as well as in the incorporation of site-specific sediment characteristics in regulatory guidelines.

**RP187 Biochemical and Molecular Responses of White Sturgeon (*Acipenser transmontanus*) to an Aryl Hydrocarbon Receptor Agonist** J. Doering, S. Beitel, Univ of Saskatchewan, Toxicology Centre; B.J. Tendler, Univ of Saskatchewan; S.B. Wiseman, Univ of Saskatchewan, Toxicology Centre; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; M. Hecker, Univ of Saskatchewan, Toxicology Centre. Dioxin-like chemicals in some sediments can bioaccumulate into fishes to concentrations sufficient to produce toxic effects. Benthic fishes such as sturgeon might be at greater risk of exposure to these chemicals. Despite their endangered status, little research has been done to characterize the relative responsiveness of sturgeon to dioxin-like chemicals. In a first attempt to characterize the biological effects and possible associated risks of exposure to dioxin-like chemicals in sturgeon we investigated the molecular and biochemical responses of white sturgeon to a model aryl hydrocarbon receptor agonist,  $\beta$ -naphthoflavone (BNF). Juvenile white sturgeon were injected intraperitoneally with BNF. Rainbow trout were used as a reference species since their responses have been well characterized in the past. Three days following injection with one of three doses of BNF (0 mg/kg, 50 mg/kg or 500 mg/kg) fish were euthanized and gill, intestine, and liver collected for biochemical and molecular analyses. In sturgeon all tissues had significantly greater ethoxyresorufin-O-deethylase (EROD) activity in exposed groups. EROD activity in liver of sturgeon exposed to 50mg/kg was 37 times greater than that of the controls, 28 times greater in the intestine, and 23 times greater in the gill. Rainbow trout had EROD activity 88 times greater than controls in the liver, but undetectable activity in the intestine and gill. EROD activity in liver of sturgeon exposed to 500mg/kg was 17 times greater than that of the controls, 51 times greater in the intestine, and 65 times greater in the gill. Methoxyresorufin-O-deethylase (MROD) activity in exposed sturgeon liver and intestine was less than EROD activity, however trout liver had activity similar to that of EROD. Based on these results, sturgeon appear to be moderately responsive to dioxin-like chemical exposure. The greater responsiveness of the intestine and gill in sturgeon suggests that these tissues might be of greater importance in the biotransformation of polycyclic aromatic hydrocarbons in sturgeon than in trout. Further research is necessary to investigate the toxicological and evolutionary significance of the observed intestine and gill enzyme activity in sturgeon.

**RP188 Bioconcentration and Bioaccumulation of Three Pharmaceuticals in Fish** S. Garcia, M. Foster, Univ of North Texas, Biology; D.B. Huggett, Univ of North Texas, Dept of Biological Sciences; L.A. Constantine, Pfizer Global Research and Development, Pharmacokinetics, Dynamics & Metabolism, Pfizer, Inc., Pharmacokinetics, Dynamics and Metabolism. Laboratory bioconcentration studies are becoming an integral part of the pharmaceutical risk assessment process. The bioconcentration factors of carbamazepine, amiodarone, and dexamethasone — three commonly prescribed pharmaceuticals in the United States — were evaluated using 42d and 7d bioconcentration factor (BCF) experiments with *Pimephales* sp. and *Ictalurus punctatus*, respectively. Further, samplings of *Oreochromis niloticus* from the Denton Wastewater Treatment Plant were made to determine a field bioaccumulation factor (BAF). *Pimephales* sp. were continuously

exposed to either a 300  $\mu\text{g/L}$  carbamazepine, a 250  $\mu\text{g/L}$  amiodarone, or a 400  $\mu\text{g/L}$  dexamethasone during the uptake phases of the BCF studies. *I. punctatus* were exposed to a 125  $\mu\text{g/L}$  carbamazepine or a 250  $\mu\text{g/L}$  amiodarone during the uptake phases. Liver, white muscle, and blood were sampled from tilapia and catfish, while only liver and white muscle were sampled from the minnows. Tissue, exposure water, and wastewater concentrations of the three pharmaceuticals were measured by isotopic dilution liquid chromatography tandem mass spectrometry (ID LC-MS/MS). The tissue specific kinetic BCFs for carbamazepine ranged from 1.3 to 71, while amiodarone BCFs ranged from 27 to 3089. Proportional BAFs for carbamazepine in the white muscle, liver, and plasma of *O. niloticus* were 2.81, 3.78, and 2.53, respectively. Dexamethasone levels in fish tissues were below detection during the laboratory and field experiments. These data will assist researchers in prioritizing future research needs with respect to individual drugs and pharmaceutical classes.

**RP189 Biomarker Responses in Sunfish and Bass from the Saluda River, South Carolina** J. Mierzejewski, Clemson Univ, Dept of Biological Sciences; D. Haney, Furman Univ; P. Van den Hurk, Clemson Univ. The Saluda River watershed, in the Upstate and Piedmont regions of South Carolina, provides drinking water for more than 500,000 inhabitants. The river also receives effluents from multiple industries, urban areas and a dozen wastewater treatment plants. In 2009 the Saluda River was ranked 6<sup>th</sup> of America's Most Endangered Rivers by American Rivers, due to its high levels of phosphorous which is assumed to mostly originate from wastewater treatment plant effluents. As the Saluda River and its reservoirs are frequently used for boating, combined with the multiple point- and non-point sources contributing to the river, it is likely that other contaminants, like PAHs, metals, pesticides and pharmaceuticals are present. Little is known about the ecological health of the river. In this study, health effects from pollutants on fish in the Saluda River were examined through the application of biomarkers. A total of 159 fish from the *Centrarchidae* family (*Lepomis* spp. and *Micropterus salmoides*) were collected during June and July 2010, at 13 sampling sites throughout the Saluda River. Here we report the first results for bile fluorescence, EROD activity, GST activity, TBARs and estrogenic compounds in bile. The highest responses were observed at the sampling sites in the center of the watershed, with lower responses seen at both the upstream and downstream ends of the river. Lake Greenwood exhibited consistently low responses. Further analyses will focus on estrogenic effects in fish and tissue accumulation of heavy metals.

**RP190 Chromium (VI) Induced Energy Allocation in *Daphnia schodleri* (Anomopoda: Daphniidae) Females and Their Offspring** M. Arzate Cardenas, F. Martinez-Jeronimo. Cladocerans accumulate energy as proteins, lipids and carbohydrates, and the amount will depend on their physiological and reproductive conditions, as well as on their age. These macromolecules are used to define the caloric content of organisms and could be modified by physical or nutritional factors or even by toxicant exposure. Aimed to evaluate how age and chromium (VI) exposure modify the caloric content of the freshwater cladoceran *Daphnia schodleri*, neonates were exposed to 6.4, 32 y 64  $\mu\text{g L}^{-1}$  of Cr ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) until they reached 5, 7, 14, 21 and 28 days old. Survivorship and fecundity were assessed during the exposure period. Neonates from different broods (at different maternal age) and reproductive females of different ages were measured; then, the caloric content of neonates and adults was assessed through macromolecules quantification and transformed by their respective combustion factor (carbohydrates 4.1  $\text{cal mg}^{-1}$ , proteins 5.65  $\text{cal mg}^{-1}$  and lipids 9.45  $\text{cal mg}^{-1}$ ) to energy content. Survivorship was significantly reduced in the group which was exposed to the higher chromium concentration but fecundity was reduced in all the exposed groups. Adults' size was negatively affected in individuals from 21 days and older. Although there were significant differences among neonates' size, they cannot be attributed to the chromium toxic effects but natural variation. Moreover, neonates produced by stressed daphnids showed higher energetic reserves, situation that could be interpreted as an energetic preparation for the adverse conditions they will cope with, even at maternal expense since adult females allocated resources for detoxification and reproduction, switching from quantity to quality offspring in terms of their energy reserves.

**RP191 Consistency of Morphological Endpoints Used to Assess Developmental Timing in Zebrafish (*Danio rerio*) Across a Temperature Gradient** A. Shaffer, M. Elrod-Erickson, R. Otter, Middle Tennessee State



Univ, Biology. Zebrafish have been used as a model toxicological system for studying developmental effects of contaminants. The combination of transparent embryonic development and a well-described genome have made this model an attractive choice for researchers interested in investigating potential teratogenic compounds. Classic methods to measure developmental delays using zebrafish have focused on the time it takes for embryos to hatch. However, this endpoint misses major developmental events during development. In this study we investigated the consistency of non-lethal developmental endpoints commonly utilized in the literature that occur prior to hatching exposed at temperatures of 24.5°, 26.5°, or 28.5° C. These endpoints were 1) time to reach a specific morphological milestone, 2) the distance between the optic cup and the otic vesicle, and 3) the distance between the optic cup and the otic vesicle divided by the diameter of the otic vesicle. The objectives of this study were to quantify the influence of temperature on each endpoint and to determine the consistency amongst endpoints. Results show a direct effect of temperature on each endpoint, with lower temperatures causing slower development. Results also show a high amount of consistency between each endpoint. Results of these studies have also led to the development of a quantifiable relationship model for each endpoint based on temperature, allowing researchers to compare results of studies at different temperatures in a more direct manner as well as development of a cost-benefit analysis of each endpoint measured.

**RP192 Coupling Ecological and Process/ISO based LCA to Assess the Production of Wood Derived "Drop-in" Biofuels** N. Bortsie-Aryee, A. Halog, Univ of Maine, School of Forest Resources (Research Group for Industrial Ecology, LCA & Systems Sustainability) With the ever increasing interest in biofuels production as a result of declining stocks of fossil fuels, energy independence, proven environmental benefits, rural economic development as well as the renewability of biomass feedstocks, it has become critically vital to examine the sustainability of the whole biofuels supply chain. Bio-oil, which is a product of recent innovations in biotechnology, genomics and bioengineering, is an oxygenated compound containing carboxyls, carbonyls and phenolics and is miscible in water. Bio-oil production involves the degradation of biomass in the absence of oxygen through the process of pyrolysis, which in the end produces a fuel which can be used for various energy intensive processes. Though the shift towards biofuels seems beneficial, an in-depth understanding of its short and long term consequences is required. This analysis should encompass the impacts starting from the supply of biomass to production of bio-oil and eventually to its consumption while considering its social acceptance, economic viability and environmental soundness. A hybrid ecological based and process-based LCA has been used to assess the environmental and ecological impacts of wood-derived bio-oil production. This will capture the impacts on ecosystem goods and services as well as the resource consumptions and associated environmental emissions during the life cycle stages. The LCA results will help scientists and policymakers to make decisions to support "drop-in biofuels" technology development in view of meeting the ideals of sustainable development. Our results will be presented and discussed in this conference.

**RP193 Critical Analysis of Read-across and Categories Under REACH: A Case Study with Xylenols** S. Erler, Smithers Viscient; C. Waites, Sabic Innovative Plastics, Staff Toxicologist; E. Mihaich, Environmental and Regulatory Resources; C. Smith, Smithers Viscient. As part of integrated testing strategies under REACH, a registrant needs to consider the potential for using read-across and the formation of categories to avoid unnecessary testing and prioritize study plans. However, the relevance of read-across and category approaches must be judged on a case-by-case basis, taking into account the quality of existing data and regulatory context in which the results are used. As a case example, this paper proposes a process for assessing and justifying groupings of xylenols and related chemical families for registration under REACH. Xylenols are a set of isomers with the same functional groups, which share toxicological properties. Previously, xylenols have been subject to groupings under the OECD Existing Chemicals HPV Programme and used for European Union hazard classification purposes. Nevertheless the reporting of groupings, as well as the assessment of extensions of groupings to structurally-related chemistry, requires a methodological approach. An examination of ecotoxicological endpoints through the combination of (Q)SARs and predicted degradation pathways is presented to illustrate the use of structural and computational methods in building a category hypothesis. Consideration is given to the advantages and disadvantages of different options for reporting in REACH registration dossiers. Finally, the potential

impact of relying on non-test data is evaluated in terms of implications to environmental hazard assessment and risk management of xylenols.

**RP194 Cyclic Volatile Methylsiloxanes (cVMS) and Selected PCB Congener Residues in Body Tissues of American Mink Obtained from Lake Pepin, Minnesota USA** K.B. Woodburn, J.A. Durham, R.M. Seston, Dow Corning Corporation, Health & Environmental Sciences; D.E. Powell, Dow Corning Corporation, Health & Environmental Sciences, Dow Corning Corporation, Health & Environmental Sciences (C03101) The concentrations of cyclic volatile methylsiloxane (cVMS) materials and selected polychlorinated biphenyl (PCB) congeners were measured in mink tissue (fat, muscle, and liver) taken from organisms collected in Lake Pepin, Minnesota, USA. This large freshwater watershed has previously been examined for the trophic magnification behavior of the cVMS compounds octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) in an aquatic food web consisting of benthic invertebrates and fish. Three male and one female mink were captured on Lake Pepin in November 2008 by a licensed trapper. The mink were dissected to remove the liver, perirenal fat, and a portion of quadriceps muscle from a rear leg. The stomach was also removed for content identification to identify dietary preferences immediately prior to death. Average cVMS concentrations in mink (lipid-adjusted) are lower than lipid-adjusted cVMS residues in Lake Pepin whole fish and benthic invertebrate organisms. Possible explanations include a continuation of cVMS trophic dilution observed in Lake Pepin fish, exhalation of cVMS by mink, cVMS metabolism, and dietary dilution with non-aquatic species in the mink diet. By comparison, lipid-adjusted concentrations of PCB congeners (e.g., PCB153 and PCB180) from mink feeding primarily on Lake Pepin aquatic organisms are equal to or greater than their respective levels in fish, benthic invertebrates, zooplankton, or sediment, consistent with the known biomagnifying properties for some PCB congeners. These data collectively indicate that Lake Pepin mink may be demonstrating continued trophic dilution of cVMS in higher trophic level species, a trend that is similar to that reported for cVMS in food webs terminating in seabirds (i.e., kittiwakes and eiders) and grey seals. Additionally, Lake Pepin mink may be demonstrating biomagnification of PCB congeners such as PCB153 and PCB180 in the aquatic food web of the lake, including mink.

**RP195 Determination of CBR Values for Three Current-use Pesticides in *Hyalella azteca*: Predictive Techniques vs. Direct Tissue Residue Measurement** B. Holzer, Oklahoma State Univ, Dept of Zoology, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology; J.B. Belden, N.L. Cooper, Oklahoma State Univ, Dept of Zoology. Extensive pesticide application has contributed to the contamination of surface water, placing aquatic organisms at an elevated level of risk of toxicant exposure. Most aquatic toxicity assessments use water or sediment chemical concentrations as a "dose metric" to indicate the level of exposure experienced by organisms in aquatic systems. Although, measurement of the amount of chemical present at the site of toxic action would result in the most accurate dose metric, such a measurement is not typically feasible. As an alternative, whole body residue (or critical body residue) analysis may provide a better estimate of effective dose. This is especially true for lipophilic, non-reactive compounds. Although the critical body residue (CBR) approach has been used in previous studies, very little body residue data is available for current use pesticides. The objective of this study was to determine the toxicity of three pesticides (bifenthrin, pendimethalin, and trifluralin) to an aquatic arthropod (*Hyalella azteca*) using three critical body residue approaches. Method 1 (using predicted BCF and empirical LC<sub>50</sub> values) and 2 (using empirical BCF and empirical LC<sub>50</sub> values) were calculated for each compound and compared to the directly measured (LBR<sub>50</sub>) tissue concentration. Results from this study indicate that both Method 1 and 2 (7.4 and 0.3 µmol/kg bifenthrin; 3.27 and 0.70 mmol/kg pendimethalin; and 0.63 and 0.87 mmol/kg trifluralin, respectively) overestimate CBR values, resulting in an underestimation of toxicity. Thus, the best measure of CBR is empirical determination during toxicity testing.

**RP196 Determination of Diethylsulfosuccinate (DOSS) in Oysters Using Single Quadrupole Mass Spectrometry** M. Miller, J. Wang, W. Schnute, Thermo Fisher. A method for analysis of sodium diethylsulfosuccinate (DOSS), the major component of Corexit dispersant, was developed and applied to both seawater and oyster samples. A HPLC system coupled with a C18 column provided separation of the analyte from the matrix. A

single quadrupole mass spectrometer provided the sensitivity and selectivity to accurately confirm identity and quantitate the target analyte. Samples of simulated seawater and oysters were spiked to evaluate the extraction efficiency and reproducibility. An automated sample extraction device employing pressurized heated solvents was used to speed the process. The extract was diluted, reducing matrix background as well as bringing the final concentration of the analyte in line with the calibrated range of the analysis. Preliminary Data: Linear response was demonstrated in standards ranging across 3 orders of magnitude from low ppb level to ppm concentrations. The method for extraction of DOSS from oysters was evaluated for efficiency of extraction as well as reproducibility. Oysters were homogenized in a blender and aliquots of the resulting emulsion loaded into the extraction cell and spiked with DOSS. A mixture of 1:1 acetonitrile and water proved optimal for analyte solubility and extraction time was minimized by heating to 100 °C and pressurizing to 1700 psi the solvents in the automated extraction instrument. Oyster homogenates were spiked in concentrations ranging from 250ppm down to 2.5ppm of DOSS. At all levels it was necessary to dilute the solvent extract to lower the concentration to levels that were consistent with the instrument calibration curve. The data collected show that the analysis in the oyster matrix was reproducible and had high efficiency for recovery. This demonstrated that the combination of procedures for the extraction and analysis is capable of measuring samples at the current FDA limit of exposure (250 ppm) and well below. Should exposure limits be reduced in the future, there exists the opportunity of reducing or eliminating the dilution of the extracts to achieve a lower reporting limit level.

**RP197 Determination of Pesticides from Water Samples in Chilean Rivers** A. Giordano; M. Retamal, Universidad de Chile, Centro Tecnológico AGQ América; J. Suarez, Centro Tecnológico AGQ América; P. Richter, Universidad de Chile. There is concern about the quality of water in ecosystems due to increased production and application of pesticides that eventually reach surface waters, making necessary to develop methodologies that can detect organic pollutants in rivers. A multi-residue method has been developed for determination and confirmation of 55 pesticides in water samples by GC-MS/MS (LODs: 0.75-19.8 ng/L). The analytes belonging to different pesticides families were determined in a chromatographic run after an extraction procedure. The method was used to make a comparison between rivers in the Metropolitan Area and in the Bio-Bio Area of Chile, due to agricultural activity. Samples were taken at three points from Mapocho/Maipo rivers (Metropolitan Area) and on a different day sample at three points of Itata/Ñuble rivers (Bio-Bio Area) were taken. The study show presence of diazinon, carbaryl, atrazine, myclobutanil and terbuthylazine in Mapocho river with concentrations between 40-95 ng/L, while in Itata river show presence of diazinon, chlorpyrifos-ethyl, tebuconazole, fenvalerate, cypermethrin and cyhalothrin in concentrations between 2.9-7.6 µg/L. Other pesticides were not detected. Different compounds has been detected in both rivers, because sampling areas have different types of crops: vegetables for Itata river and vineyard/fruit for Maipo river. The difference in concentrations observed may be due to application dates and methodology: in Itata rivers pesticides in vegetables are applied nearest to the soil.

**RP198 Differences in Seasonal and Precipitation Patterns for Two Fecal Bacterial Indicators** M. Sirzlar, Middle Tennessee State Univ, Molecular Biosciences, Texas A&M Univ, Wildlife and Fisheries; S. Winesett, M. Garmon, M. Barbero, Metro Water Services; F. Bailey, Middle Tennessee State Univ, Dept of Biology *Escherichia coli* (*E. coli*) is the main fecal bacterial indicator (FIB) under the Clean Water Act used to classify waterbodies as impaired for pathogens. One major limitation of *E. coli* as an indicator is the inability to associate fecal sources to their warm-blooded host, causing difficulty in applying appropriate watershed management strategies. *Bacteroides* spp., a potential alternative FIB, has been shown to be highly host specific for human, equine, and bovine 16S rRNA gene targets. It is imperative to look at long-term monitoring data to establish life histories and patterns of FIB under varying conditions to direct regulatory agencies for future source tracking protocols. Past data collected by Metro Water Services, Nashville, TN, USA, found *E. coli* concentrations (colony forming units) to increase during the summer months (June, July, Aug) and to decline substantially in the winter season. We collected both *E. coli* and *Bacteroides* in baseflow and wet weather conditions from March 2010-April 2011 for sixteen impaired streams located in Nashville, TN. Colilert and qPCR methods were used for detection of *E. coli* and *Bacteroides*, respectively. Seasonal and weather differences existed for both *E. coli* and *Bacteroides* ( $p < 0.001$ ). *E.*

*coli* concentrations were higher in wet weather than baseflow scenarios, but *Bacteroides* concentrations did not differ between baseflow and rainfall samples. *E. coli* was statistically higher ( $p < 0.001$ ) in the summer and *Bacteroides* tended to higher in the winter. These results may supplement EPA's water quality criteria development and direct potential seasonal use or seasonal-specific criteria of FIBs. Watershed managers may also find this beneficial for optimal source-tracking conditions.

**RP199 Early Life-stage Exposure to Selenium by Egg Injection of Pallid Sturgeon (*Scaphirhynchus albus*) and Shovelnose Sturgeon (*S. platyrhynchus*)** D.M. Papoulias, US Geological Survey, Columbia Environmental Research Center; M. Annis, D. Nicks, US Geological Survey; D.E. Tillitt, US Geological Survey, Columbia Environmental Research Center; M. Schwartz, US Fish and Wildlife Service. Endangered pallid sturgeon and congeneric shovelnose sturgeon found in the lower Platte River, NE and elsewhere in the Missouri River basin have elevated tissue levels of selenium. Their piscivorous-omnivorous diet and long period of recrudescence between spawns facilitates Se bioaccumulation into eggs and hence offspring exposure. Early-life stages of fishes (embryo-larva) are sensitive to Se effects and are thought to be a significant bottleneck limiting sturgeon populations. Therefore, the objective of this study was to determine what concentrations of Se affect survival of young *Scaphirhynchus* sturgeon. Embryos were injected with graded doses of selenomethionine (SeMet nominal doses: 0, 6, 10, 18, 32 µg Se/g dry egg wt) within 24h post-fertilization (PF) to simulate maternal exposure to selenium. Developmental effects were evaluated at 7d PF. Mortality was assessed at 17d PF and mortality and growth were evaluated at 32d PF. Genotoxic effect of Se was evaluated at 32d PF by erythrocyte micronuclei. Actual mean Se concentrations in embryos immediately after injection were estimated to be 9, 12, 17, 23 µg Se/g egg and 6, 7, 13, 17 µg Se/g egg for shovelnose and pallid, respectively. Background egg concentrations were 5 µg/g egg and 3 µg/g egg for shovelnose sturgeon and pallid sturgeon, respectively. Developmental deformities (delayed development, edema, and skeletal deformities) were mostly observed within the first 7d PF at a nominal dose > 10 µg Se/g egg. A higher percentage of pallid larvae than shovelnose larvae presented with developmental deformities and these were lethal. In general, mortalities were highest within the first 17d PF. Shovelnose mortality was significant at estimated doses of 17 and 23 µg/g egg. Pallid sturgeon mortality was significant at the highest estimated dose tested of 17 µg Se/g egg. However, overall pallid survival was low for all doses and time points and fertilization rate for pallid eggs was lower than for shovelnose eggs, suggesting that poor pallid sturgeon egg quality may have confounded results. Growth was not significantly affected in either species. Although the magnitude of the effects differed between the species, the effective selenium concentrations and the associated types of deformities were similar. The results of these studies will assist biologists in determining whether selenium toxicity is a factor limiting recovery of the pallid sturgeon in some parts of its range.

**RP200 Ecological Bioavailability of Permethrin and *p,p'*-DDT: Toxicity is Dependent on Type of Organic Matter Fraction** J.D. Maul, Texas Tech Univ, The Institute of environmental and human health, The Institute of Environmental and, Dept of Environmental Toxicol; A.J. Trimble, Ashland Univ; M.J. Lydy, Southern Illinois Univ, Fisheries and Illinois Aquaculture Center and Dept of Zoology. Allochthonous leaf litter is a primary source of carbon into stream ecosystems, providing a linkage of terrestrial and aquatic systems and a major energetic resource for stream food webs. Leaf litter is fractionated into different components through biological processing (primarily leaf shredding by invertebrates and decomposition) and is important for cycling of organic carbon and stream ecosystem functioning. Use of allochthonous litter inputs drives differentiation of aquatic invertebrate communities into distinct functional feeding groups. These inputs are also a major adsorptive substrate for hydrophobic contaminants; an interaction that occurs from minimally decomposed coarse particular organic matter to very fine organic matter that is suspended or dissolved. This suggests that contaminant distribution may not be homogeneous within stream systems and could be more concentrated in certain locations or resource bases within streams. Likewise, resource utilization among stream macroinvertebrates is not homogenous; leading to the hypothesis that there may exist disproportionate contaminant effects among invertebrate taxa that is dependent upon how and where they use litter-based resources (i.e., bioavailability driven by ecological function). Here, we test this hypothesis by: (1) measuring partitioning of permethrin and *p,p'*-DDT among leaf-based organic matter



fractions and sediments, (2) examining variation in *Hyalella azteca* toxicity among permethrin and *p,p'*-DDT contaminated sediment, leaf, and a mixture, and (3) using SPME to understand bioavailability of freely dissolved fractions in leaf and sediment treatments. *H. azteca* lethal concentrations for 50% of the population (LC<sub>50</sub>s) for permethrin and *p,p'*-DDT were lowest in the leaf exposure and highest in sediment for permethrin and sediment-leaf mixture for *p,p'*-DDT. Log K<sub>oc</sub> values for both permethrin and *p,p'*-DDT were greatest within sediment and lowest in coarse leaf material. The SPME results indicated that both compounds had the highest levels of bioavailability in leaf material, lowest levels in sediment, and intermediate levels in the mixtures. These three lines of evidence demonstrate that toxicity, sorption, and bioavailability of permethrin and *p,p'*-DDT varied among organic matter fractions common within stream ecosystems, and supports the idea that ecological traits are important for driving the risk of contaminant impacts in stream systems.

**RP201 Ecotoxicity of Textile dye C.I. Acid Orange 7 and Studies in Advanced Oxidative Process** L.A. Luna, Campinas State Univ, LEAL – Laboratory of Ecotoxicology and Environmental Microbiology, Campinas State Univ; T.H. Silva, R.F. Nogueira, Paulista State Univ, Dept of Analytical Chemistry; F. Kummrow, Federal Univ of Sao Paulo, Dept of Exact Sciences and Earth; G.A. Umbuzeiro, Campinas State Univ, LEAL – Laboratory of Ecotoxicology and Environmental Microbiology. Dyes have been a concern in freshwater pollution. These substances may reach the water bodies and the exposure may cause toxicity to aquatic organisms and also inhibit photosynthetic process due to light absorbance competition. In that way textile effluents containing dyes must have their color and toxicity removed. The aim of this work was assess the toxicity of the textile azo dye C.I. Acid Orange 7 to *Daphnia similis* as well the effectiveness of photo-Fenton process to degrade this azo dye and remove the toxicity. The assays were carried out according to OECD and the acute toxicity was determined by EC50-48h. The C.I. Acid Orange 7 was obtained with 85% of purity (Sigma®). The EC50-48h to *Daphnia similis* was 86.0 mg.L<sup>-1</sup>, and thus C.I. Acid Orange 7 can be considered harmful to aquatic life according to Global Harmonized System of Classification and Labeling of Chemicals. In a laboratory scale reactor using UV-vis artificial irradiation and Fenton's reagent (Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>), 500 mL of C.I. Acid Orange 7 solution (60.0 mg.L<sup>-1</sup>) was submitted to the treatment. The degradation of the azo dye and the formation of the byproducts were monitored using high performance liquid chromatography. We have performed toxicity assays in 5, 8 and 30 minutes of treatment to assess the toxicity of byproducts generated. In 60 mg.L<sup>-1</sup> of untreated dye was observed 10% of immobility. After 5 minutes of treatment 90% of immobility was observed, in 8 minutes 10% and after 30 minutes no immobility was observed. The acute toxicity of 5 minutes effluent sample was higher than the original dye which was not completely degraded according to the chromatographic analysis, thereby these byproducts may be responsible to the toxicity increase. On the other hand, 8 minutes effluent sample showed the same percentage of immobility of untreated dye, however after 30 minutes of treatment the toxicity was removed and the C.I. Acid Orange 7 was completely degraded. The next steps of this research include acute and chronic toxicity assessment to species from different trophic levels and chemical identification of toxic byproducts. Acknowledgements: Financial support, FAPESP – Thematic Project (2008/10449-7) and Master scholarship (2010/12991-3).

**RP202 Effect of Herbicide Mixtures on Microbial Communities from Prairie Wetlands: A Whole Wetland Approach** S. Sura, Univ of Saskatchewan, Food and Bioproduct Sciences; M.J. Waiser, Environment Canada, Water Science and Technology Directorate; V. Tumber, Environment Canada, Water Science and Technology Directorate; P. Messing, Univ of Manitoba, Ph. D. Candidate; A. Farenhorst, Univ of Manitoba, Associate Professor. The wetlands in the Prairie pothole region of Saskatchewan and Manitoba serve an important role in providing wildlife habitat, water storage and water filtration. These wetlands are regularly interspersed among intensive agricultural operations where herbicides and insecticides with multiple active ingredients are commonly used. Mixtures of herbicides and insecticides are often detected in prairie wetlands. A whole-wetland approach has been used to investigate the effects of herbicide mixtures (2,4-D, MCPA, clopyralid, dicamba, dichlorprop, mecoprop, bormoxynil, and glyphosate) on wetland microbial communities. The herbicide mixture at the recommended field application rate had a significant effect on the phytoplankton productivity of both pelagic and biofilm communities over relatively short

time period. Stimulatory effects of auxin-type herbicides in the mixture as a result of 'concentration addition' was evident on primary production. BI-OLOG and pigment profiles suggested change in the community structure. The herbicide mixture appears to have affected not only the microbial communities but also invertebrates (higher trophic level) suggesting a resonance effect of changes in the microbial communities.

**RP203 Effect of UV Filter Benzophenone-4 on Planktonic Crustacea** S. Yoon, S. Nam, Konkuk Univ; Y. An, Konkuk Univ, Dept of Environmental Science. UV filters are widely used in sunscreens to block ultraviolet light. Benzophenone-4 is an ingredient of sunscreens, and is frequently detected in water body. In this study, the acute toxicity of UV filter benzophenone-4 was evaluated using three crustacea to investigate how these common aquatic species were influenced by exposure to benzophenone-4. Test species were *Daphnia magna*, *Moina macrocopa*, and *Simocephalus mixtus*. Acute assay was conducted according to OECD TG No.202, and survival and immobilization were measured. All tests species were adversely affected by benzophenone-4, and the 24h-EC50 values for all species were in the range of 247 to 303 mg/L showing similar sensitivity. *Daphnia magna* tended to be more sensitive to benzophenone-4 than *Moina macrocopa* after 48h exposure. Further studies are needed to investigate the effect of other kinds of UV filters in aquatic ecosystem.

**RP204 Effects of 17 $\alpha$ -Ethinylestradiol (EE2) on Northern Pike (*Esox lucius*)** S. Beitel, S. Wiseman, J. Raine, M. Hecker, Univ of Saskatchewan, Toxicology Centre. Exposure to environmental estrogens and other endocrine-active chemicals have been shown to impact reproduction of freshwater fish species. One environmental estrogen of particular concern is the synthetic estrogen 17 $\alpha$ -Ethinylestradiol (EE2), which has 10–50 fold greater potency than natural estrogens. While extensive research has been conducted regarding the interaction of this chemical with model laboratory and some European fish species, little is known about the potential effects of environmental estrogens to freshwater fish species native to North American environments such as northern pike (*Esox lucius*). Pike are ubiquitous in water bodies throughout North America and Europe, and are potentially at risk of exposure to elevated levels of environmental estrogens e.g., in waters downstream of waste water treatment facilities. Therefore, the aim of the present study was to investigate effects of semi-chronic waterborne exposure of northern pike to a model estrogen, EE2, and to compare the responses to those previously reported for standard laboratory model species. Pike were exposed to three concentrations of EE2 (0ng/L, 5ng EE2/L, 100ng EE2/L) for 6 weeks. Blood, liver, gonad, head kidney, and brain were sampled. Blood plasma was analyzed for vitellogenin (Vtg) and concentrations of sex steroid hormones (testosterone, 17 $\beta$ -estradiol) were measured. These results were compared with results previously reported for other species to assess the pike's sensitivity to this chemical, and enable a reliable risk assessment for environmental estrogen exposure. Further characterization of alterations of molecular and biochemical endpoints along the hypothalamus-pituitary-gonad axis as well as histopathological changes in the gonads and liver are ongoing in an effort to establish a comprehensive understanding of the potential impacts of estrogen exposure to northern pike.

**RP205 Effects of Dietary Selenomethionine Exposure on Repeat Swim Performance, Metabolic Rate and Energy Metabolism in Adult Zebrafish (*Danio rerio*)** J. Kallarakavumkal Thomas, Univ of Saskatchewan, Toxicology Centre, Univ of Saskatchewan, graduate student; D. Janz, Univ of Saskatchewan, Toxicology Centre, Univ of Saskatchewan, Veterinary Biomedical Sciences. Selenomethionine (Se-Met) is the dominant form of selenium (Se) present in food. In a previous study we reported impaired swimming and elevated stored energy (triglycerides and glycogen) in adult zebrafish after chronic sublethal dietary Se-Met exposure. In the present study, we investigated effects of chronic sublethal dietary Se-Met exposure on repeat swimming performance, oxygen consumption (MO<sub>2</sub>), cost of transport (COT) and energy metabolism in adult zebrafish. Adult fish were fed varying concentrations of Se (1, 3, 10 and 30  $\mu$ g Se/g, dry weight) in the form of Se-Met for 90 days. At the end of the exposure period, fish from each treatment group were divided into three subgroups: a) no swim, b) swim, and c) repeat swim. Fish from the no swim group were euthanized immediately at 90 days and whole body triglycerides and glycogen were determined. Individual fish from the swim group were placed in a swim tunnel respirometer and swim performance was determined using the critical swimming speed (Ucrit) method. After both Ucrit and MO<sub>2</sub> analyses, fish



were euthanized and whole body energy stores were determined. Similarly, individual fish from the repeat swim group were subjected to two Ucrit tests (Ucrit-1 and Ucrit-2) performed with a 60 min. recovery period between tests, followed by determination of energy stores. Impaired swim performance was observed in fish fed greater than 3 µg Se/g in the form of Se-Met, however no differences were observed between Ucrit-1 and Ucrit-2 within each treatment group. Both resting and active metabolic rates (MO<sub>2</sub>) and COT were significantly greater in fish fed elevated dietary Se-Met. Whole body triglycerides and glycogen increased with increasing dietary Se-Met exposure. Fish from all treatment groups were able to mobilize stored glycogen during repeat swimming, and a similar trend was observed for triglycerides in all except 3 and 10 µg Se/g fed groups. Our ongoing studies are investigating mRNA expression of key enzymes (e.g., fatty acid synthase, 3-hydroxy-acyl-CoA-dehydrogenase and citrate synthase) involved in energy metabolism in liver and muscle. Overall results from this study should provide a better understanding of mechanism(s) underlying altered energy homeostasis in fish exposed to Se-Met.

**RP206 Effects of Environmentally Relevant Triclocarban Exposure Concentrations on Survival, Growth and Shredding Activity of the Amphipod *Hyalella azteca*** D. Edwards, Texas Tech Univ, The Institute of environmental and human health; J.D. Maul, Texas Tech Univ, The Institute of environmental and human health, The Institute of Environmental and, Dept of Environmental Toxicol. Triclocarban (TCC) is a common antimicrobial agent added to a wide array of household and personal care products such as soaps, toothpastes, lotions, and deodorants. In 2010, the EPA estimated annual mass of TCC produced in the US was 226,796 kg. Due to its primary use in household and personal care products, the vast majority enters the WWTP cycle and ultimately the environment via effluent and bio-solids applications. Contamination of US streams is widespread and TCC entering the environment through treatment plant effluent has been measured at low µg/L concentrations. Despite the use of TCC in household products for over 45 years, there are few studies regarding its toxicity to aquatic organisms and a disparity of information when compared to other common antimicrobial compounds such as triclosan. Of research published to date, the vast majority concentrates on the fate and persistence of TCC. Our research explores both the chronic and acute effects of TCC on the amphipod *Hyalella azteca*, a leaf shredding macroinvertebrate. Shredding macroinvertebrates are critical to activities throughout the stream continuum and effects on shredding of allochthonous leaf litter has the potential to disrupt a wide range of downstream processes and ecological functions. Preliminary results indicate that TCC is acutely toxic to *H. azteca* at environmentally relevant concentrations. Additionally, our results indicate that relevant concentrations of TCC inhibit growth and feeding behavior of *H. azteca*. *H. azteca* exposed to TCC for 96 hours displayed an LC50 of 4.8 µg/L TCC. *H. azteca* exposed to treatments of 1.25 µg/L TCC and 2.5 µg/L consumed 8.5 % and 12 % less leaf material than controls. *H. azteca* exposed to concentrations as low as 0.15 µg/L TCC were significantly smaller, in length, than controls. Results from additional experiments will be discussed. This work will be used as a basis to perform further analysis on the effects of environmentally relevant concentrations of TCC on shredding macroinvertebrates and their ability to process leaf material as a critical process in the stream continuum.

**RP207 Effects of Kingston Fly Ash on Freshwater Mussels** R. Otter, Middle Tennessee State Univ, Biology, Middle Tennessee State Univ, Dept of Biology; W. Monroe, Middle Tennessee State Univ, Dept of Biology. On December 22, 2008 the largest release of fly ash in United States history occurred at the TVA Kingston Fossil Plant in Roane County, Tennessee. The result was contamination of the Emory River and surrounding area. Efforts to understand the toxicological implications of this spill are currently underway with investigations into the impact of fly ash on a wide range of biota. In this study we investigated the impact of Kingston fly ash on freshwater mussels, both a field and laboratory setting. Our field study consisted of up to a year exposure, in situ, using multiple native freshwater mussel species at both upstream and downstream sampling sites, compared to the location of the spill. Endpoints used to determine impact include survival, a mussel health index, and total glycogen content. Results were species dependent, but overall, all species showed a high survivability throughout the entire exposure period. Laboratory studies utilized a custom static recirculating tank design to expose the freshwater mussel *Urbicula imbecilis* to varying concentrations of Kingston fly ash. Survival and glycogen concentrations

were measured and compared directly to the treatment concentration of fly ash.

**RP208 Effects of Metal Exposures on the Antioxidant Status and Tissue Damage in Eastern Oysters, *Crassostrea virginica*** B. Khan, M. McCarthy, Univ of North Carolina at Charlotte, Biology; A.H. Ringwood, Univ of North Carolina at Charlotte, 9609 C Vinca Circle. Aquatic ecosystems are exposed to multiple stressors simultaneously. Many estuarine sites which show diurnal dissolved oxygen and pH fluctuations are also exposed to metals which can be toxic to marine organisms. Bivalve mollusks serve as good indicators of metal pollution in estuarine ecosystems. It is known that exposure to these metals can affect the antioxidant status of the tissues and can damage membrane lipids as well as cause DNA damage and modification in marine organisms. The overall purpose of this study was to identify the relationship between tissue metal concentrations and antioxidant status and to estimate oxidative damage in Eastern oysters, *Crassostrea virginica*. Oysters were collected from polluted and relatively clean sites (including NC oyster sanctuary sites), and metal concentrations were measured in gill and hepatopancreas tissues. Glutathione and lipid peroxidation levels were measured as markers of overall antioxidant status and tissue damage respectively. Laboratory studies were also conducted wherein oysters were exposed to metals dissolved in seawater. The same biomarkers were measured to determine the effects of metal exposures on antioxidant status and tissue damage. While oysters from the uncontaminated sites had no evidence of significant tissue damage and showed fairly stable glutathione levels, increased tissue damage and changes in antioxidant status were observed at higher concentrations of dissolved metal. The sites with high fluctuations in dissolved oxygen and pH as well as high concentration of metals showed increased tissue damage and disruption of the antioxidant status in bivalves. The interactions between these stressors are important determinants of the health of oyster populations in estuarine ecosystems.

**RP209 Effects of Municipal Wastewater Effluent on Reproductive Function in Wild Fish Exposed in an Effluent Dominated Stream** G.R. Terreault, Univ of Waterloo, Biology; J. Bennett, Environment Canada, National Water Research Institute; M.R. Servos, Univ of Waterloo, Dept of Biology and Canadian Water Network; M.E. McMaster, Environment Canada, National Water Research Institute. Studies by Environment Canada on Wascana Creek in Regina, Saskatchewan, Canada, have determined that during the winter low flow periods the creek is 100% treated sewage making it a model system to understand the effects of MWW. In this study, we were interested in determining whether MWW effects reproductive development in fish in Canada, and if so, does the impairment influence the survival of fish populations. In 2007 and 2008, field studies assessed sentinel fish species under pre-spawning, spawning and post-spawn conditions in terms of growth (condition factor), and reproduction (in vitro sex steroid capacity, vitellogenin, gonadosomatic indices, and histology). Comparisons were made in fish collected upstream and downstream of Regina's Wastewater Treatment Plant. Abundant species in this system are the fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*). Both sexes of spawning fathead minnow collected downstream of the sewage discharge were smaller, had reduced condition and larger liver somatic indices when compared to fish collected upstream of Regina. MWW exposed males also had elevated levels of vitellogenin and lower scores of secondary sexual characteristics (fewer nuptial tubercles, little or no development of the dorsal pad, and no dorsal fin dot). Exposed female stickleback had increased gonad size and corresponding increased testosterone production but decreased 17 $\alpha$ -estradiol productive capacity compared to reference fish. Other alterations in histopathology observed included inflammation of tubules in the kidney and stunted gill lamellae. Post-spawning collections revealed an absence of sufficient mature fathead minnows downstream of the MWW discharge for site comparisons, although stickleback were abundant and did not demonstrate any significant site differences in performance.

**RP210 Effects of Pharmaceutical-Fungicide Mixtures on Shredding Detritivore Responses Under Varying Temperature Regimes** M. Willming, Texas Tech Univ, The Institute of environmental and human health; J.D. Maul, Texas Tech Univ, The Institute of environmental and human health, The Institute of Environmental and, Dept of Environmental Toxicol. Pharmaceuticals and fungicides have been detected in the aquatic environment, and their presence may have implications for bacterial and fungal communities and the invertebrate detritivores responsible for the decomposition

of allochthonous organic matter, a major energy source for many aquatic ecosystems. Few studies have examined how mixtures of antibiotics and fungicides could impact decomposer-detritivore systems. Additionally, in some aquatic systems daily water temperature fluctuations may influence these processes and alter contaminant toxicity, but such diurnal temperature regimes are rarely examined in conjunction with contaminants. In this study *Hyalella azteca* served as a model shredding detritivore and organisms were exposed to pharmaceuticals, triclosan and ciprofloxacin, and fungicides, chlorothalonil and propiconazole, at near environmentally relevant concentrations of compounds both individually and in mixtures. Experiments were performed at a constant temperature, diurnal temperature regime, and a diurnal temperature regime adjusted based on future climate predictions for south-central Texas. Endpoints included survivorship, growth and leaf processing. Results indicated a significantly lower 10-d LC<sub>50</sub> for organisms exposed to chlorothalonil at a diurnal temperature [LC<sub>50</sub> 95% confidence interval) = 70.4 µg/L (63.7 – 79.5)] compared to organisms exposed to chlorothalonil under a constant temperature [LC<sub>50</sub> = 80.1 µg/L (71.6 – 92.3)], based on the LC<sub>50</sub> ratio test. There was a trend toward increased leaf processing at higher concentrations of chlorothalonil. Additional results will be presented. Ultimately, effects on aquatic decomposition processes due to such compounds may impact energy and nutrient flow through the aquatic food web, affecting multiple trophic levels. This work will also provide information on the influence of realistic temperature scenarios on contaminant effects in aquatic systems, which is an important initial step for understanding how future temperature alteration due to climate change may influence the assessment of ecological risk of contaminants.

**RP211 Effects of Predator Cues on Pesticide Toxicity: Toward an Understanding of the Mechanism of the Interaction** G. Qin, Texas Tech Univ, The Institute of Environmental and Human Health and Dept of Environmental Toxicology, Texas Tech Univ; S. Presley, T. Anderson, W. Gao, Texas Tech Univ; J.D. Maul, Texas Tech Univ, The Institute of Environmental and Human Health, The Institute of Environmental and, Dept of Environmental Toxicol. Pesticide toxicity may be modified by a number of co-occurring environmental and ecological stressors. Coexposure to predator cues has been shown to potentiate and/or synergize toxicity of pesticides. However, the mechanisms behind these interactions are not well understood. Here we examine the effects of fish predator (bluegill, *Lepomis macrochirus*) cues on toxicity of five different pesticides to the freshwater cladoceran, *Ceriodaphnia dubia*. The purpose for examining patterns among pesticides was to test the idea that the mechanism of the interaction could be explained by a general stress response; that is, the interaction patterns would be similar regardless of the pesticide's mechanism of action (MOA). Acute 96-h concentration–response experiments were conducted for pesticides with and without fish cues. Predator cues influenced the toxicity of pesticides and the interaction patterns varied among pesticides. Fipronil exhibited a synergistic interaction, while predator cues interacted antagonistically for bifenthrin and thiacloprid. Other compounds previously reported to potentiate toxicity (malathion) were found to act additively. The results demonstrate that factors such as pesticide bioavailability, KOC, and exposure concentration may be important for predicting the occurrence of these interactions and that patterns were not consistent among pesticides varying in MOA. Predator stress is an important component for structuring communities and ecosystem processes. Fully understanding how this process may interact with organic contaminants may best be achieved by examination at toxicokinetic and toxicodynamic scales.

**RP212 Effects of Pulp and Paper Discharge in Largemouth Bass: An Evaluation of Effects Across Multiple Levels of Biological Organization** T. Gross, Environmental Resource Consulting, Dr. Timothy S. Gross, Univ of Florida; S. Holm, Georgia-Pacific Corporation, Environmental Affairs; O. Burgess, Univ of Florida, Fisheries and aquatic Sciences, Environmental Resource Consultants. Largemouth bass (*Micropterus salmoides*) have been utilized to assess the effects of exposure to pulp and paper discharge at Georgia Pacific's Palatka mill since 1998 as a model to assess the effects of mill process upgrades. These efforts have focused on controlled treatments and natural exposures, as well as effects at the organism and lower biological levels (i.e., biochemical). Results demonstrate dose dependent effects at the biochemical level (i.e., decreased plasma sex steroids), tissue and cellular level (i.e., decreased GSI), and organism level (i.e., decreased reproductive status) at high exposure scenarios, however, effects for bass exposed under natural conditions are less than predicted from dilution profiles alone. The

translation of these effects to higher levels of biological organization were unclear from these original assessments. Current efforts have focused on an assessment of effects at higher levels of biological organization, including population and community levels. Assessments include the collection of largemouth bass across several sites within the lower St Johns River basin to reflect a wide range of exposures. Collections were conducted across 28 sites and four times annually: January, April, July and October. Measures included: catch per unit effort; sex ratio, size/age distribution; body weight, length and condition index; reproductive status; and biomarkers of reproductive function (i.e., plasma sex steroids, and Vtg). Results demonstrate diminished effects and dose responses with the implementation of process upgrades at the organism level and below, as well as significant increases in animal numbers and catch per unit effort for high exposure sites. Results across all sites demonstrate clear seasonal and annual variance that must be characterized and considered in any assessments of population/community effects. Significant differences were also detected for bass populations as a function of habitat type: natural littoral zones and man-made structure littoral zones, however, similar annual and seasonal trends were noted across habitat types. Differences in body weight, length and condition index; sex ratio; and size/age distributions were not detected regardless of pulp-and-paper exposure levels. Reduced bass numbers and decreased catch-per-unit effort was noted at the highest exposure level, however, this is likely due to smaller stream size and different habitat traits.

**RP213 Effects of Retene Exposure on Mentum Deformity Incidence in Laboratory Reared *Chironomus riparius* Larvae** A.K. Karjalainen, Univ of Jyväskylä, Dept of Biological and Environmental Science; K. Vuori, Finnish Environment Institute; M. Leppanen, Univ of Eastern Finland; H. Hamalainen, Univ of Jyväskylä. Alkylphenanthrenes such as retene (7-isopropyl-1-methylphenanthrene) are commonly found at elevated concentrations in sediments downstream of pulp and paper mills. Retene toxicity in fish has been suggested to be mechanistically similar to that of dioxins. In rats and humans developmental dental aberrations induced by dioxins (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) are mediated via binding to cytosolic Ah receptor. High incidence of mouthpart deformities, especially mentum teeth aberrations, have been observed in midge (*Chironomidae*) larvae inhabiting sediments polluted by pulp and paper mills. Since Ah receptors homologous to vertebrate receptors exist in invertebrates, we hypothesized that retene may also increase the incidence of mentum deformity in midge larvae. We exposed laboratory reared *Chironomus riparius* larvae 14 days to retene spiked sediments at ecologically relevant concentrations of 248.0±67.4 µg/g sediment dry weight (mean±SD), and used the proportion of individuals with a deformed mentum (DI %) as a toxicity endpoint. Further, types and degree of mentum deformities and impact of varying organic carbon concentration of sediment were analysed. Our treatments included 900 individuals that were fed with 1.2 mg fish flake food suspension per larva on days 0 and 7. Unlike in field populations of *Chironomus* larvae, our laboratory animals seem to have high DI % not only in contaminated but also in the control sediments. Concurrently measured larval growth was not affected by the lowest content of 1.4±0.1 % organic carbon of sediment dry weight (mean±SD) alone but was slightly decreased in coexposure to retene. Larval survival in retene-exposures and control treatments was slightly lower in sediment with the lowest carbon content when compared to sediment with the highest carbon content of 23.1±0.1 % of dry weight (mean±SD). Our results suggest no specific receptor-induction of retene in *C. riparius*. Problems related to identification of mentum deformities are discussed.

**RP214 Effects of Soluble Epoxide Hydrolase Inhibitors on Cardiac Function and Embryonic Development in Zebrafish, *Danio rerio*** N. De La Paz, Univ of California, Riverside, Dept of Environmental Sciences; S. Hwang, B. Hammock, Univ of California, Davis, Dept of Entomology and Cancer Center; D. Schlenk, Univ of California Riverside, Dept of Environmental Sciences. Epoxyeicosatrienoic acids (EETs) are endogenous substrates for soluble epoxide hydrolases (sEHs), which catalyze the formation of dihydroxyeicosatrienoic acids (DHETs). DHETs have critical roles in cardiac development and inflammation within vertebrates. Previous studies have shown that phenyl ureas inhibit sEHs in multiple species. Thus, to better understand the roles sEH and DHETs play in vertebrate development, zebrafish embryos were exposed for 72 h to 0.5, 5, and 50 nM of several sEH inhibitors, 1-(1-acetypiperidin-4-yl)-3-adamantanyleurea (APAU), 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU, *trans*-4-[4-(3-Adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB),



and trans-4-[4-[3(4-Trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy]-benzoic acid (*t*-TUCB). Morphology, survival and embryonic heart rate were determined for each embryo at 48, 58, and 72 hours post fertilization (hpf). After 48 hours, the highest concentrations of APAU, TPPU, and *t*-AUCB, diminished heart rate from  $81.1 \pm 0.3$  (beats/30sec) in controls to  $65.2 \pm 1.9$  (APAU),  $74.9 \pm 2.3$  (TPPU), and  $76.4 \pm 0.3$  (*t*-AUCB). After 48 hours exposure to 5 and 50 nM TPPU curvature of the tail was observed in 30 and 23% of the embryos, respectively. Embryo lethality (28% and 60%) was also observed following exposure to 5 and 50 nM TPPU. These results indicate that inhibition of sEH by several distinct structures may deleteriously affect early development in zebrafish although other mechanisms should also be explored.

**RP215 Effects of the Biopesticide Candidate *Metarhizium anisopliae* S54, on the Crustacean *Ceriodaphnia dubia*** X. Chen, Univ of Lethbridge, Dept of Geography; J.L. Dan, Univ of Lethbridge, Geography. Environment and health concerns about the using of chemical insecticides to reduce large-scales insect pest infestations have led to renewed interest in the development of entomopathogenic fungus for pest control and environmental ecosystem protection. *Metarhizium anisopliae* is a hyphomycetous fungus that is pathogenic to some insect pests. A strain *Metarhizium anisopliae* S54 was isolated from a soil in Alberta, Canada, and has been under development and field testing in 2008-2010. This present study was conducted to assess potential pathogenesis of this fungus to a non-target cladoceran species, *Ceriodaphnia dubia*. Acute relative mortality and population responses to chronic exposure of a commercial agriculture R-11, the chemical pesticide neonicotinoid imidacloprid, biopesticide Azatin and suspended conidia of *Metarhizium anisopliae* S54 on the crustacean, *Ceriodaphnia dubia* were examined. For the acute study, *Ceriodaphnia dubia* was exposed to 0, 0.25 mg/l ( $1.33 \times 10^4$  conidia/ml), 0.5 mg/l ( $2.66 \times 10^4$  conidia/ml), 1 mg/l ( $5.33 \times 10^4$  conidia/ml), 2.5 mg/l ( $1.33 \times 10^5$  conidia/ml), 5 mg/l ( $2.66 \times 10^5$  conidia/ml) and 10 mg/l ( $5.32 \times 10^5$  conidia/ml), with the inoculum being replaced every 24h until 144h. For the chronic population study, *Ceriodaphnia dubia* populations were exposed to Expected Concentration (EEC) levels of the agricultural adjuvant R-11 (0.79 mg/l), chemical pesticide neonicotinoid imidacloprid (0.0174 mg/l), biopesticide Azatin (0.033 mg/l) and suspended conidia of viable *Metarhizium anisopliae* S54 (0.033 mg/l) for 8 days. The number of founding individuals, offspring/female, final population size, and population growth rate were assessed in treated and control groups. Acute relative mortality ranged from 0% in 0.25 mg/l ( $1.33 \times 10^4$  conidia/ml) concentrations to 5% in 10 mg/l ( $5.32 \times 10^5$  conidia/ml) concentrations after 144 h treatment. Exposure to imidacloprid caused greater reductions in the production of offspring per female and population size parameters than the other compounds, at the rates tested. The agricultural adjuvant R-11, biopesticide Azatin, and spores of S54 were less challenging to *Ceriodaphnia dubia*, and are not expected to pose any significant risk to aquatic organisms, as represented by the test organism *Ceriodaphnia dubia*.

**RP216 Effects of Water Chemistry on the Toxicity of Cu to the Fish Parasite *Ichthyophthirius multifiliis*: Development of a BLM for Therapeutic Application** A.C. Ryan, HDR|HydroQual; D. Straus, USDA/ARS, Harry K. Dupree – Stuttgart National Aquaculture Research Center; J. Tomasso, Texas State Univ. *Ichthyophthirius multifiliis* (Ich) is a common ectoparasite that invades the skin and gills of freshwater fish. Copper sulfate ( $\text{CuSO}_4$ ) is often used to control infestations of Ich in pond aquaculture in the United States. In this study, we determined the acute toxicity of  $\text{CuSO}_4$  to the free-swimming theronts of Ich in reconstituted waters. Water chemistry characteristics, including calcium, magnesium, and pH were varied independently and spanned relevant ranges. The objective of these experiments was to provide data that would serve as the basis for development of a biotic ligand model (BLM) that could be used to derive therapeutic doses of copper sulfate in aquaculture operations. A BLM for such purposes would be ideal because it could define effective bioavailability-based doses for controlling Ich outbreaks while simultaneously evaluating if copper doses would be safe for fish. Preliminary data analysis suggested that solution chemistry influenced the toxicity of  $\text{CuSO}_4$  to Ich, and that Ich strain or batch contributed to the variability in the observed effect concentrations. Development of a BLM for Ich may have to be strain-specific, which for aquaculture purposes suggests that the least sensitive Ich strain should be used for BLM development.

**RP217 Elucidating the Toxicity and Sub-cellular Fate of Inorganic Selenium in Four Marine Microalgae** R.R. Ronchin, K.M. Damian, C.L. Smith, D.F. Jolley, Univ of Wollongong. Selenium (Se) has been associated with a range of toxic effects in aquatic and terrestrial organisms. Currently there is minimal information regarding the physiological response of microalgae to Se. Microalgae are at the base of the aquatic foodweb, hence any accumulated Se may be transferred to higher trophic organisms. This research investigated the toxicity of two inorganic species of Se to the marine microalgae *Dunaliella tertiolecta*, *Nitzschia closterium*, *Phaeodactylum tricornutum* and *Tetraselmis* sp. The algae were exposed to selenite ( $\text{Se(IV)}$ ) and selenate ( $\text{Se(VI)}$ ) under environmentally relevant conditions.  $\text{Se(VI)}$  was significantly more toxic than  $\text{Se(IV)}$ , with  $\text{Se(VI)}$  exposures producing mean 72-h  $\text{IC}_{10}$  values (concentrations that inhibited population growth (IC) by 10%) ranging from 1.2 to 7.6 mg/L for *P. tricornutum* and *D. tertiolecta*, respectively, whereas  $\text{Se(IV)}$   $\text{IC}_{10}$  values ranged from 53 to 150 mg/L, respectively. To determine the intracellular level and distribution of Se, cells were exposed to  $\text{IC}_{10}$  levels of  $\text{Se(VI)}$  for 72-h, harvested, washed, and analysed by ICP-MS. *D. tertiolecta* had the greatest intracellular Se with up to  $119 \times 10^{-15}$  g Se/cell, compared to  $< 10 \times 10^{-15}$  g Se/cell for the other microalgae. The sub-cellular distribution (method of Lavoie et al., 2009) of accumulated Se was primarily associated with the organelle and cytosol (~78%), with less Se in the cell debris (~20%) and granule (~2%) fractions for *D. tertiolecta*, *P. tricornutum* and *N. closterium*. However, *Tetraselmis* sp. had the greatest proportion of Se associated with the cell debris fraction (~55%), followed by the organelle and cytosol (~30%) and granule fraction (~4%). Although multiple cell disruption conditions were investigated, it was not possible to rupture algal cell membranes without significant organelle disruption, as confirmed by citrate synthase activity. Hence, identifying the Se in the metabolically sensitive cytosol (i.e., separation of the organelle and cytosol fractions during ultracentrifugation) could not be performed. This demonstrates that  $\text{Se(IV)}$  and  $\text{Se(VI)}$  are not toxic to these microalgae at concentrations typical of a contaminated marine environment. Future work should investigate methods to rupture these algal cell membranes without compromising organelle integrity, and repeat these tests using chronic exposures at environmentally significant selenium concentrations. Lavoie, M., S. Faucheur, et al. (2009). Aquatic Toxicology 92: 65-75.

**RP218 Endocrine Disrupting Effects of Six Major Organophosphate Flame Retardants in H295R and MVLN Cells, and Zebrafish** X. Liu, k. Ji, k. choi, Seoul National Univ. Flame retardants have been widely used in construction materials, furniture, plastics, electronic equipments, textiles, and other materials. Because of the ban of polybrominated diphenyl ethers (PBDEs), production and use of organic phosphate flame retardants (OPFR) has increased. Although OPFRs have been detected in the house dust, surface water, fresh water biota, human urine and milk at the levels similar to or greater than those of PBDEs, understanding of potential health effects are limited. We evaluated endocrine disrupting potencies of six major OPFRs including tris-(2-chlorethyl) phosphate (TCEP), tricresyl phosphate (TCP), tris-(2-butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), tris-2-chloroisopropyl phosphate (TCPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCPP) using H295R and MVLN cell lines as well as adult zebrafish. In H295R cell line, steroid hormone production and expression of mRNAs that are responsible for steroidogenesis and estrogen metabolism were measured. In fish test, sex hormones and mRNAs of steroidogenic enzymes were determined after 14 d exposure of 4 m old zebrafish to three OPFRs that were identified to be potent in vitro evaluation. With MVLN cell line, estrogen receptor binding activities of the OPFRs were evaluated. In H295R cell, all six OPFRs could induce both estradiol (E2) and testosterone (T) production. In addition, all major steroidogenic genes like 3 $\beta$ HSD, CYP19 were up regulated except CYP17, and sulfotransferase genes including SULT2A1, SULT2B1 and SULT1E1 were downregulated. In MVLN cell, TPP, TCP, and TDCPP could inhibit binding of estrogen to estrogen receptor. In 14 d fish exposure, 0.2 mg/L TCP, or 1 mg/L TDCPP and TPP significantly increased plasma T and E2 concentrations in females, while T decreased and E2 increased in males. The gene transcription of CYP17 and CYP19 was significantly up regulated in both sexes, while VTG was down- and up-regulated in female and male fish, respectively. Exposure to test OPFRs generally lead to feminization of male and masculinization of female fish. Both in vitro and in vivo studies showed that OPFR could affect steroidogenic pathway and alter sex hormone balance. Responses of male and female fish to these chemicals were different, and underlying mechanisms deserve further study.



**RP219 Endocrine Disruption or Adaptation? Loss of an Acute Cortisol Stress Response with Chronic Copper Exposure in Rainbow Trout** M.S. Tellis, D. Alsop, C. Wood, McMaster Univ, Dept of Biology. The aim of this study was to determine the effects of chronic waterborne Cu exposure on stress-induced acute cortisol release and the associated physiological implications in rainbow trout (*Oncorhynchus mykiss*). Trout were exposed to 30 µg Cu/L in moderately hard water (120 mg/L as CaCO<sub>3</sub>) over a period of 40 d, following which time, they were challenged with a series of stressors. At 40 d, an approximate 60% increase in Cu levels were observed in the gill and liver, but not in the tissues of the hypothalamus-pituitary-interrenal axis (HPI axis). Fish exposed to Cu did not increase circulating cortisol levels with 1 min air exposure or 24 h isolation, in contrast to controls which increased cortisol by 3-fold. However, this inhibitory effect on cortisol mobilization appeared to have few implications on the ability of Cu-exposed fish to maintain homeostasis. For example, plasma Na<sup>+</sup>, Ca<sup>2+</sup> and glucose levels as well as hepatic glycogen levels were the same post-stress in control and Cu-exposed fish. Trout were also challenged with an exposure to 50% seawater for 48 h, where Cu-exposed trout maintained plasma Na<sup>+</sup>, glucose and hepatic glycogen levels. However, Cu-exposed fish experienced decreased plasma K<sup>+</sup> levels throughout the Cu exposure and stress tests. In conclusion, chronic Cu exposure resulted in the abolition of an acute cortisol response after stressor exposure. This did not appear to be due to a toxic effect of Cu on the HPI axis, and did not affect the ability of the fish to maintain ion and carbohydrate homeostasis. The lack of an acute cortisol response may be a coping strategy to reduce costs during the chronic stress of Cu exposure, and not endocrine disruption as a result of toxic injury. (NSERC Strategic Grant, Rio Tinto Alcan, Environment Canada).

**RP220 Environmental Hazard Assessment of Petroleum Distillates** J.P. Swigert, EcoTox Assessments, LLC; C. Lee, ExxonMobil Biomedical Sciences, Inc.; D.C. Wong, Shell Oil Company – Shell Health; P. Podhasky, American Petroleum Institute. Assessing the environmental hazards of petroleum substances has historically been challenging for these difficult to test substances. The establishment of USEPA and OECD High Production Volume (HPV) chemicals programs compiled new and existing data that provided information necessary to assess the environmental and human health hazards of many chemicals and chemical categories. Where data gaps were identified, testing was conducted to progress robust hazard assessments of these complex petroleum substances. This presentation summarizes the experimental ecotoxicity and biodegradability results for three petroleum categories characterized by the boiling point/hydrocarbon continuum: gasoline (exemplified by principal carbon range of 4 – 12); kerosene (carbon range 9 – 16), and gas oil middle distillates (carbon range 9 – 25). Owing to their variable and complex compositions, petroleum substances possess a wide range of physical-chemical attributes. Aquatic toxicity test results are presented for different petroleum substances using the water accommodated fraction (WAF) preparation technique for exposure solutions. The WAF method of creating exposure solutions allows the ecotoxicity of a substance to be expressed in terms of the amount of product required to produce a particular effect. Other exposure methods such as dilutions of water soluble fractions are not comparable to tests using WAFs because they do not allow the multi-component dissolution behavior of the complex substance to be considered. Similarly for biodegradability testing, many different test methods exist; however, not all are appropriate for testing petroleum substances. Therefore biodegradation data were developed using a test method recommended for volatile substances having low water solubility. Results from these studies provide a reliable basis for characterizing environmental hazard across these distillation fractions and were found to be consistent with model predictions inferred from detailed composition analyses of these streams.

**RP221 Evaluating the Joint Toxicity of Lead and Cypermethrin to the Benthic Invertebrate, *Chironomus dilutus*** W.T. Mehler, Gradient; J. Du, J. You, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry; M. Lydy, Southern Illinois Univ Carbondale, Fisheries and Illinois Aquaculture Center and Dept of Zoology. Insecticides and heavy metals are frequently detected in the environment, but few studies have assessed the joint toxicity of organic and inorganic contaminants. Joint toxicity of a pyrethroid insecticide, cypermethrin and a heavy metal, lead was evaluated in the current study. An antagonistic toxic response was observed when the benthic invertebrate, *Chironomus dilutus* were simultaneously exposed to the two contaminants

in both water and sediment exposures. Pre-exposure bioassays with midges were also conducted and toxicity of cypermethrin was significantly reduced to midges which were pre-exposed to lead. Additionally, the effect of lead on the bioavailability of cypermethrin to midges was measured using Tenax extraction. No significant difference was noted in the amount of Tenax-extractable cypermethrin when different amounts of lead were added to sediment. Results suggested that altered organism sensitivity may have contributed to the observed antagonistic interaction between cypermethrin and lead, while changes in toxicokinetic processes, such as uptake and biotransformation, on the joint toxicity should be further researched.

**RP222 Evaluation of Whether Gemfibrozil is a Peroxisome Proliferator in Fish** S.Y. Skolness, Univ of Minnesota, Dept of Biochemistry and Molecular Biology; E. Durhan, K.M. Jensen, M. Kahl, USEPA, Mid-Continent Ecology Division; C. LaLone, USEPA; E. Makynen, D.L. Villeneuve, USEPA, Mid-Continent Ecology Division; G.T. Ankley, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology Division. Gemfibrozil is a pharmaceutical that indirectly modulates cholesterol biosynthesis through effects on peroxisome proliferator-activated receptors (PPAR), which are transcriptional cofactors that regulate expression of genes related to lipid metabolism. An enzyme found in the peroxisome known to have its activity regulated by PPAR activation is fatty acyl-coenzyme-A oxidase (FAO). Although, gemfibrozil is a known mammalian hepatic peroxisome proliferator, it may not operate via this mechanism in non-target species such as fish. Because gemfibrozil is commonly detected in surface water, it is important to investigate the effects of the drug on non-target species. The purpose of this study was to determine if gemfibrozil elicits the same pharmacological mechanism in fish as it does humans, by measuring in vitro effects of gemfibrozil on FAO activity in fish. In vitro experiments with liver tissue explants from fish species (fathead minnow and zebrafish) were incubated with various concentrations of gemfibrozil and FAO activity was measured. Initial results suggest that gemfibrozil is a less potent peroxisome proliferator than in mammals, and may not operate via the same pharmacological mechanism in fish as in humans. This study adds to our knowledge of possible effects of a common aquatic pharmaceutical pollutant on fish PPAR actions. The views expressed in the current study are those of the authors and do not reflect the views or policies of the US Environmental Protection Agency, nor does mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**RP223 Exposure to Persistent Organic Pollutants Through Ingestion of Marine Debris: The Relevance of Partition Coefficients** J. Newsted, C. Berger, D. Kay, J. Phillips, Cardno Entrix. This paper examines the relevant published literature regarding potential marine organism exposure to persistent organic pollutants (POPs) through incidental ingestion of plastic debris. While plastics have the capacity to absorb chemical substances from their surrounding environment, little is known about the factors effecting adsorption and desorption of POPs to plastic debris, the abundance and bioavailability of POPs on plastic debris, and the amount of plastics marine organisms consume. The first step in understanding these dynamics is an evaluation of relevant partition coefficients. Partition coefficients can be used to understand the fate of compounds in the environment and to evaluate the relative contribution of POPs adsorbed to plastics and the total exposure of marine organisms to POPs in the environment. Therefore, we conducted an extensive review of relevant partition coefficients found in the peer-reviewed literature. Since marine organisms will be found in an aqueous environment, we then focused on water to plastic partition coefficients ( $K_{pw}$ ). The results of our review identified  $K_{pw}$ s for 18 specific types of polymers and 203 individual compounds representing 12 different chemical groups. Analyses of these data showed a wide range of values (e.g., 2.14 to 7.09 log  $K_{pw}$ ) among compounds for any given polymer. Of note, our correlation analysis showed a relationship between log  $K_{pw}$  and log  $K_{ow}$  (octanol-water partition coefficients) for most polymers. Consequently, log  $K_{ow}$  may act as a surrogate for estimating log  $K_{pw}$ , which will allow for extrapolation (i.e., modeling). This finding is of particular importance because of the lack of log  $K_{pw}$  (water partition coefficient) data for a wide array of plastics. A logical next step is to use log  $K_{ow}$  to generate log  $K_{pw}$ s through modeling of a wide range of plastics in order to understand the potential for these plastics to adsorb POPs in then environment. Our review of the primary literature is an initial step towards understanding partitioning coefficients and more information is

needed from other sources (plastics companies, federal/state agencies, etc.) to garner a substantiated critical review.

**RP224 Forms of Sexual Abnormalities in the Fiddler Crab, *Uca pugnator*** E. Zou, Nicholls State Univ, Biological Sciences. Like other brachyuran crustaceans, fiddler crabs, *Uca pugnator*, are normally gonochoristic and exhibit striking sexual dimorphism. Males are characterized by a pair of extremely asymmetrical chelipeds and a narrow abdomen while females typically have a pair of small, identical claws and a broad abdomen, which virtually covers the entire sternum. Recently several sexually abnormal fiddler crabs have been discovered, representing four different forms of sexual abnormalities. All these crabs were obtained from the Gulf Specimen Marine Laboratories, Inc. of Panama Florida. At present, it is still unknown whether there is an environmental etiology behind these sexual aberrations.

**RP225 Fractionation of Cu in Aquatic Foodweb** K.Y. Garcia, Univ of Texas El Paso, Biological Sciences; J. Navarrete, Univ of Texas El Paso, Geological Sciences; E. Walsh, Univ of Texas El Paso, Biological Sciences; D. Borrok, Univ of Texas El Paso, Geological Sciences. As an aquatic pollutant, copper has received considerable attention. The toxicity of copper has been demonstrated for a number of algal and rotifer species. Further it has been shown that exposing rotifers to copper in the presence of an algal food source decreases its toxicity. This affect has been detected both when algae are present during copper exposure and when rotifers are fed pre-exposed algae. To better understand the interaction between copper toxicity and trophic transfer, we investigate how copper (Cu) may be fractionated by cellular processes occurring in a simple food web consisting of a grazer, the rotifer *Brachionus plicatilis*, and its alga food source, *Tetraselmis suecica*. Algae were grown in Cu-citrate enriched media to simulate forms of organic bond Cu dissolved in natural waters and fed daily to rotifers for nine days. Algae were harvested daily while rotifers were collected 0, 5, and 8 days after algal inoculation and rinsed three times to remove any residue Cu. Samples were then digested with 70% nitric acid and ultrapure 30% hydrogen peroxide in preparation for ICP-OES analysis of Cu concentration and isotopic analysis. Preliminary results demonstrated that the algae assimilated the heavier  $\delta^{65}\text{Cu}$  isotope while the rotifer internalized the lighter  $\delta^{63}\text{Cu}$  isotope at day 0. However, as time increased rotifers assimilated the heavier isotope from the isotopically heavy algae. These findings, along with those from an earlier study investigating copper uptake by bacteria, may indicate that at lower trophic levels organisms preferentially uptake  $\delta^{65}\text{Cu}$  while primary consumers incorporate the heavier isotope through their diet. Copper mass balances are being calculated and additional experiments will be conducted with freshwater species to confirm these results. Our ultimate goal is to link Cu isotope changes with toxicological indicators such as reduced population growth rates and expression of metal regulating proteins.

**RP226 Habitat Effects on Mercury Bioaccumulation in Black Sea Bass (*Centropristis striata*)** G. LeBlanc, Roger Williams Univ, Marine Biology; M. Soto, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología, Roger Williams Univ, Marine Biology. Mercury (Hg) is a widespread environmental contaminant that bioaccumulates in fish muscle tissue, and therefore poses a risk to human consumers. Understanding the human risk from Hg exposure requires insight into: (1) species-specific Hg concentrations and (2) variability in fish Hg content as a function of life history (e.g., habitat use and diet). In this study, an important recreational fish, the black sea bass (*Centropristis striata*) was collected from inshore (Narragansett Bay Estuary) and offshore (Rhode Island-Block Island Sound; RIS-BIS) habitats using trawls and hook & line. The white muscle tissue of black sea bass ( $n = 36$ ) was analyzed for total Hg and results were evaluated relative to fish age and habitat use (inshore vs. offshore). The otoliths of the inshore sea bass population were analyzed for strontium (Sr) concentrations to detect evidence of a salinity signature, and thus, a verification of site fidelity in the Bay and RIS-BIS. Visual analysis of stomach contents was also performed to assess variation in diet across habitats, which could account for geographic differences in sea bass Hg contamination. Irrespective of habitat-type, the Hg content of black sea bass muscle tissue was positively correlated with fish age, indicating the bioaccumulation of Hg. Black sea bass collected from inshore habitats, however, had higher Hg levels at a given age than conspecifics from offshore locations. Further, within the inshore habitat, individuals with a lower Sr concentration (lower salinity signature) had higher Hg levels. The cumulative results

indicate that Hg concentrations in black sea bass vary significantly over relatively small spatial scales (5 km), and site-specific sea bass Hg levels are correlated with the anthropogenic contaminant sources in the Narragansett Bay. Finally, the diet of the inshore sea bass population was dominated by crabs (50% frequency of occurrence, FO), whereas offshore conspecifics fed on crabs (28% FO), shrimp (24% FO), and algae (16% FO). Future work will include the analysis of black sea bass stable isotope signatures and otolith microchemistry (for offshore fish), as well as examining the Hg content of sea bass preferred prey across habitats.

**RP227 Hazard Assessment of Inorganic Selenium Under REACH: A Pragmatic Approach** P. Van Sprang, ARCHE; N.M. Deleebeeck, Arcadis Belgium, REACH & Product Stewardship Services; K. Oorts, ARCHE. Selenium is an essential element showing a very narrow margin between dietary essentiality and toxicity. So far, the main focus of the extensive research on selenium has been the environmental fate and effects of the element in the aquatic environment. The available scientific knowledge indicates that diet is the primary exposure pathway for both aquatic invertebrates and vertebrates and that selenium toxicity is primarily manifested as reproductive impairment in egg-laying vertebrates (fish and birds) due to maternal transfer. The severity of the observed adverse effects appears to be more related to tissue concentrations than to aquatic concentrations of the element and selenium toxicity seems to be largely species- and site-specific. Therefore, it is generally accepted that a single predicted no effect concentration (PNEC) based on aqueous Se concentrations would not be appropriate for protecting against selenium toxicity in all systems and that selenium requires site-specific risk assessment to a much greater extent than many other contaminants. However, the European REACH Regulation and other regulatory risk assessment methods traditionally require the derivation of a single threshold concentration of a substance for each environmental compartment (e.g., water, soil, sediment) based on toxicity data for the most sensitive species and ecosystem. For selenium, such an approach would entail the risk of defining PNEC values within the deficiency range for many systems. Since REACH and similar regulations are pushing the limits with regard to timely submission of chemical safety reports, they also force difficult substances such as selenium to be assessed in a pragmatic way using the scientific knowledge already available. A pragmatic approach for the aquatic and terrestrial hazard assessment of selenium under REACH will be presented and the potential for further refinement will be discussed.

**RP229 How Does Life History Affect Concentrations of PCBs in Arctic Food Fishes?** H. Swanson, Univ of Alberta; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology. Arctic char (*Salvelinus alpinus*) and lake trout (*Salvelinus namaycush*) are an important subsistence food source for northerners in the North American Arctic. In coastal Arctic regions, both Arctic char and lake trout have plastic life histories and may adopt either an anadromous (sea-run) or lake-resident life cycle in response to environmental and endogenous cues. Some populations of Arctic char and lake trout are in fact partially anadromous; in these populations, some fishes migrate to sea whereas others do not. There are no genetic differences between sympatric anadromous and resident (i.e., freshwater) fishes, but we have recently shown that mercury concentrations are significantly lower in anadromous fishes than in resident fishes. This appears to be because anadromous fishes have faster growth rates and higher lipid contents than resident fishes. We are currently investigating how life history affects concentrations of polychlorinated biphenyls (PCBs) in Arctic char and lake trout. It is possible that, similar to mercury, PCB concentrations are lower in anadromous fishes due to growth dilution. PCB concentrations may also be higher in anadromous fishes, however, because of relatively higher lipid contents. In this study we investigate the effect of life history on PCB concentrations in Arctic char and lake trout, and we evaluate how northern subsistence fishers may alter their exposure to both mercury and PCBs by targeting different life history types of these fishes.

**RP230 Hydrocarbons, Fluorescent Bile Metabolites and MMCs in Hardhead Catfish (*Ariopsis felis*) to Assess Impact in the Coast of Tabasco, Mexico** N. Ramirez-Miss, Cinvestav Unidad Merida, Marine Resources Dept; G. Gold-Bouchot, Cinvestav Unidad Merida, Marine Resources. To evaluate the environmental impact of the oil industry in the coast of the Mexican state of Tabasco (Southern Gulf of Mexico), 80 Hardhead Catfish (*Ariopsis felis*) were captured and analyzed. Petroleum hydrocarbons (including PAHs, aliphatics and UCM) in liver, fluorescent metabolites in bile, and



melanomacrophage centers (MMCs) in spleen were determined, as well as total and standard length and weight. After accounting for fish size (standard length or weight), the correlation of hydrocarbon concentrations or bile metabolites with MMCs was assessed. Results for the use of MMCs as an indicator for coastal management are discussed.

**RP232 In Vivo and In Vitro Effects of Chlorpyrifos in Grass Shrimp (*Palaemonetes pugio*) and Mysids (*Americamysis bahia*)** P. Key, NOAA/ National Ocean Service; E. Simonik, Ohio Univ; N. Kish, Univ of South Carolina; M. Fulton, NOAA/National Ocean Service, Marine Ecotoxicology Division. Organophosphate insecticides (OPs) cause toxicity by inhibiting acetylcholinesterase (AChE) in the nervous systems of both vertebrates and invertebrates. This enzyme catalyzes the hydrolysis of the neurotransmitter, acetylcholine (ACh), from the synaptic cleft. When AChE is inactivated by the binding of an OP, ACh accumulates at the nerve synapse and interferes with normal nervous system function. This quickly leads to twitching of the voluntary muscles, paralysis, and death. Chlorpyrifos is an OP insecticide that continues to be used worldwide in agricultural and residential settings, though in 2001 its use in the United States was greatly restricted. The goal of this study was to assess the in vivo and in vitro effects of chlorpyrifos in two model estuarine crustaceans, the grass shrimp (*Palaemonetes pugio*) and the mysid (*Americamysis bahia*) and compare their sensitivities. Grass shrimp are abundant in estuaries along the Atlantic and Gulf Coasts. They play a vital role in the breakdown of detritus and serve as an important food source for commercially and recreationally important fish species. Mysids also play an important role in estuarine food webs and both crustaceans are widely used in toxicity testing. Results from the in vitro experiments indicated that the target enzyme in the two species was similar in sensitivity to OP-induced inhibition with  $IC_{50}$ s of  $10^{-8.94}M$  and  $10^{-9.78}M$  for mysids and grass shrimp, respectively. In vivo experiments, however, showed that mysids were significantly more sensitive to chlorpyrifos-induced AChE inhibition after 24h of exposure. The  $EC_{50}$ s for AChE inhibition were 0.27  $\mu g/L$  for mysids and 13.35  $\mu g/L$  for grass shrimp. The significance of these findings as they relate to chlorpyrifos-induced mortality in each species will be discussed.

**RP234 Influence of Pesticide Exposure on Dermo Infection and Stress-related Responses in the Eastern Oyster (*Crassostrea virginica*)** P. McClellan-Green, North Carolina State Univ, Ctr for Marine Sciences & Technol. *Perkinsus* sp. is a pathogenic protozoan that is the causative agent of the lethal oyster disease Dermo. Oysters infected with this parasite suffer a reduction in shell growth, emaciation, reduced reproductive output and death. Oysters in many coastal habitats, including some in North Carolina, are infected with this dangerous parasite yet very little is known about the interaction between the parasite, host defense mechanisms and stress induced by anthropogenic sources. It has been shown previously that environmental parameters such as temperature stress, salinity and hypoxia suppress the ability of oysters to resist infection by *Perkinsus marinus*. Complicating this picture is the additional influence of anthropogenic factors such as pesticides on the host oyster's ability to mount an immune or defense response. Our study assessed the impact under normal environmental conditions of the 3 widely used pesticides--atrazine, chlorpyrifos and glyphosate--on the ability of oysters to resist Dermo infection. Duplicate sets of oysters were exposed to increasing concentrations (0, 10, 50 or 100 mg/L) of pesticides for 8 days. All oysters were sacrificed on day 8 and the level of infection was determined along with a number of physiological responses including expression of Hsp70 and oxidative stress. Our results indicate that glyphosate exposure results in a 2.5 fold increase in Dermo infections compared to atrazine or chlorpyrifos exposure. The levels of phenoloxidase activity following exposure were not predictive of Dermo infection. In addition, the levels of heat shock protein (Hsp 70) expression varied throughout the treatments and were not indicative of pesticide exposure.

**RP235 Interactive Effects of Phosphorus and Copper on *Hyaella azteca* and Periphyton** M. Li, Univ of Michigan, School of Natural Resources and Environment, Harvard School of Public Health, Environmental Health; D. Costello, Univ of Michigan, School of Natural Resources & Environment; A. Burton, Univ of Michigan, School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research. Eutrophication is known to be frequently associated with metal pollution in aquatic ecosystems. Our research examined the interaction between waterborne copper and phosphorus, with respect to their effects on

growth of a freshwater amphipod *Hyaella azteca* in the presence of periphyton. The study design included two tiers: (1) a laboratory stream experiment where natural periphyton communities accumulated Cu under a gradient of Cu and P concentrations (2) a beaker experiment where *H. azteca* fed on the periphyton previously grown in laboratory streams. There was rapid Cu accumulation by periphyton and the total Cu concentration of periphyton was unrelated to the dissolved P treatment. An interaction between Cu and P was observed for *Hyaella* growth where high phosphorus concentration was related to reduced growth at relatively lower Cu concentration. Our findings suggest that eutrophication may result in greater Cu toxicity to benthic macroinvertebrates as a result of dietary exposure from periphyton.

**RP236 Laboratory and In Situ Exposures of *Macrocystis pyrifera* (Giant Kelp) Sporophylls Prior to Zoospore Release for Toxicity Testing** K. Flocken, A. Cabor, Nautilus Environmental; C. Stransky, AMEC Earth & Environmental. Under the current EPA germination and germ-tube growth test method for *Macrocystis pyrifera* (Giant Kelp), zoospores are released from the sporophylls (reproductive kelp blades) within 24 hours of blade collection, and then directly exposed to a reference toxicant or effluent for 48 hours. Because the zoospores are microscopic, it is extremely difficult to conduct real time in situ exposures which can be used to better characterize dynamic environments (i.e., stormwater plume). In this study, sporophylls were exposed to either copper chloride or raw seawater for a period of 48 hours prior to zoospore release. The initial pilot study included kelp blades exposed to copper in the laboratory setting, and the zoospores subsequently released into laboratory seawater. Follow up studies using SEA-Ring™ technology developed by SPAWAR and in collaboration with AMEC Earth & Environmental repeated this method and were expanded to include in situ blade exposure to the receiving environment at UCSD's Scripps Institution of Oceanography. Zoospores from the field exposed blades were ultimately released into a copper chloride dilution series in the laboratory and allowed to grow-out for 48 hours. Results indicate that density of spore release, germination rate, and germ-tube mean length of zoospores from field-exposed sporophylls show a typical dose response to increasing concentrations of copper. Additionally, laboratory and field exposures exhibited similar sensitivity to one another. The study confirms that in situ exposure of *Macrocystis pyrifera* for toxicity testing is possible at the sporophyll level and may have future implications in stormwater or other projects that may benefit from in situ monitoring methods.

**RP237 Latent Effects of Triclosan on Development and Survival in Fathead Minnows, *Pimephales promelas*** J. Salierno, Fairleigh Dickinson Univ, Dept of Biological Sciences; M. Lopes, M. Rivera, Fairleigh Dickinson Univ. Triclosan (TCS) is an antimicrobial compound used in personal care products and enters the aquatic environment through waste water treatment plant effluent. The goal of this research was to evaluate TCS exposure on hatching, growth, and development in the fathead minnow *Pimephales promelas*. We conducted a 10d static-renewal LC bioassay in which 24hr, fertilized, eggs were exposed to 6 concentrations of TCS (0-500ppb) in triplicate, including ethanol controls. The resultant LC<sub>1</sub> and LC<sub>10</sub> (50 and 100ppb) were then used to examine sub-lethal exposure of TCS on juvenile minnow growth and development. We exposed 24hr, fathead minnow eggs to 50 and 100ppb TCS, with ethanol controls, for 10d followed by a 6-week depuration period (N = 4). Development, mortality, and deformities were monitored daily and growth (total length and wet weight) was quantified at the end of the 6-week depuration period. The 10d static-renewal LC<sub>50</sub> was 149.67ppb, which was lower than previously reported for fathead minnows. The 10d, sub-lethal, TCS exposure had no effect on the time to develop, hatch, or on larval survival compared to the controls (p = 0.84). However, differences in minnow growth and deformities were observed. TCS exposed fish after the 10d exposure to sub-lethal concentrations exhibited increased emaciation and spinal deformities compared to control fish (p = 0.08). Interestingly, there was a decrease in survival of the TCS exposed fish after the 6 week period, with the 100 ppb exposure treatment having significantly lower rates of survival than both the 50 ppb and control treatments (p = 0.004). This reduction in survival displayed a dose response by TCS concentration when compared to controls. No deformities were observed after the 6-week depuration and the significant mortality in exposed fish confounded the length and weight results, which were not analyzed. Mortality was likely a result of spinal deformities reducing foraging ability, leading to emaciation and death. The observed effects occurred at concentrations below the published LC values for TCS exposure in fathead minnows. Results suggest



that TCS exposure may exhibit deleterious effects on fish species at lower concentrations, over longer durations, than previously reported. Further, the observed mortality rates in TCS exposed fish 6-weeks after a 10d exposure demonstrates the need for various exposure paradigms to investigate the effects of TCS on fish.

**RP238 Life-Cycle Exposure of *Daphnia magna* to Environmentally Relative Mixtures of Pharmaceuticals** D.N. Wolfe, S. Richards, Univ of Tennessee at Chattanooga; M. Hanson, Univ of Manitoba. Due to the global detection of pharmaceuticals in surface water, risk assessments require toxicity testing to be performed to close knowledge gaps for effects assessment regarding impacts on aquatic food web dynamics. The widespread use of pharmaceuticals has resulted in mixture concentrations of mg/l in effluent and ug/L concentrations in surface water. Their potential toxicological effect on fresh water ecosystems remains largely unknown, especially as complex mixtures. Toxicity data on the effect of pharmaceuticals has expanded in the past decade, but has mainly focused on single, acute pharmaceutical exposures, not environmentally realistic mixtures. By determining the threshold of response for environmentally relevant mixtures, the risk that these compounds may pose to the environment will be determined more accurately. In 2008, thirteen pharmaceuticals were quantified in the Tennessee River, USA and its tributaries, ranging from 0.1757 to 0.0028 ug/l. Using the same ratios of individual compounds, but increased concentrations, the present study conducted chronic life cycle toxicity test on the cladoceran *Daphnia magna*. Tests were performed using 10x, 100x and 1000x the concentrations detected in the Tennessee River, resulting in a total concentration of 6.031, 60.31, and 603.1 ug/l of total pharmaceutical exposure, respectfully. Mortality, time to first brood, size and fecundity were used as endpoints of toxicity. Test solutions were renewed three times a week and neonate removed each day until the conclusion of the experiment. *D. magna* showed a statistically significant decrease in the number of neonate produced when exposed to 100x and 1000x ( $p < 0.003$ ). When *D. magna* was exposed to the 10x concentration, no significant decrease in neonate production was observed. Neither time to first brood nor size was affected at 10x, 100x, or 1000x. Studies are on going to pinpoint the threshold of effects occurring between 10x and 100x. These data will provide risk assessors more accurate data when analyzing non-lethal effects of pharmaceutical mixtures on aquatic ecosystems.

**RP239 Measured Mercury Contamination in Freshwater Fish in Rhode Island Compared with Predictions from a Regional Environmental Mercury Model** A. Kuhn-Hines, J. Lake, J. Serbst, USEPA, Atlantic Ecology Division; D. Nacci, USEPA, ORD, NHEERL, Atlantic Ecology Division, USEPA, NHEERL, Atlantic Ecology Division; P. Edwards, A. Libby, Rhode Island Dept of Environmental Management, Division of Fish and Wildlife. Edible tissue of largemouth bass collected at 29 freshwater sites across the variable landscape of Rhode Island, USA showed a 27 fold range in total mercury concentrations [Hg], from 0.04 to 1.0 ppm (wet). Twenty-one variables, including water quality data and geographic information system (GIS) layers, were obtained to describe the land use, human population density, soil and bed rock characteristics, impervious surfaces and vegetative cover within the watersheds and one hundred meter buffer zones surrounding these freshwater sites. Regression analyses were performed to determine which landscape and water quality variables or combinations of these variables were associated with size-corrected [Hg] in largemouth bass from the 29 freshwater sites. Preliminary analyses demonstrated that three variables: pH, chloride and Secchi depth or clarity (all negatively associated with fish [Hg]), explain 58% of the variability in largemouth bass [Hg] among sites. Measured [Hg] results were compared with those estimated for the same sites using the MERGANSER model, a USGS-EPA New England (NE) regional model which uses mercury depositional data and estimated values for continuous water quality and landscape variables to predict [Hg] in fish. Average measured values of fish [Hg] for these 29 lakes were generally similar to predicted values (0.50 and 0.48 ppm, respectively), while variance was slightly higher in the measured data set. Fish [Hg] measured and predicted values were moderately well correlated ( $r^2 = 0.40$ ), and rank order agreed better for lakes with the lowest and highest fish [Hg] values. The pH value measured at sites, which was not used in calculating MERGANSER model estimates of [Hg] because this information was not available for many NE lakes, could explain a higher % of the variability in the model's estimates of [Hg]. Our results suggest that easily measured water quality variables such as pH, chloride and Secchi depth data could provide important information

for the prediction of fish [Hg] in unmeasured lakes. Further, these findings indicate more site specific modeling may be required in regions like Rhode Island where site characteristics vary widely across a relatively small geographic range.

**RP240 Mechanism for Disruption of Crustacean Molting by Xenobiotics** E. Zou, Nicholls State Univ, Biological Sciences. Agricultural and industrial activities have led to aquatic pollution of various anthropogenic chemicals. Some of these xenobiotics, such as organochlorines, can readily accumulate in aquatic crustaceans. A series of laboratory exposure studies have shown that several environmental xenobiotics are capable of disrupting crustacean molting, with most of the disrupting effects being molt-inhibitory. Initial in vivo mechanistic studies using N-acetyl- $\beta$ -glucosaminidase (NAG), also known as chitinase, as a biomarker for ecdysteroid signaling have revealed that xenobiotics inhibit crustacean molting through disturbing the Y-organ-ecdysteroid receptor (EcR) axis. A further analysis utilizing tissue culture and quantitative real-time PCR techniques has demonstrated that the disturbance of such an endocrine axis by certain xenobiotics involves disruption of ecdysteroid signaling in the epidermis. When molt-inhibiting agent Aroclor 1242, 2,4,5-trichlorobiphenyl (PCB29), endosulfan or kepone was administered alone, the expression of NAG gene in cultured epidermal tissues was upregulated, while heptachlor had no effects. Under binary exposure to both 20-hydroxyecdysone and a molt-inhibiting chemical, a condition similar to the natural hormonal milieu of epidermal tissues of animals impacted by such a chemical, both Aroclor 1242 and endosulfan were found to be capable of antagonizing ecdysteroid signaling in cultured epidermal tissues. This antagonizing effect on epidermal ecdysteroid signaling can at least partly explain the inhibitory effects of these two agents on crustacean molting. PCB29, when given together with 20-hydroxyecdysone, produced an additive effect on epidermal ecdysteroid signaling but such an additive effect was not observed when kepone was combined with 20-hydroxyecdysone.

**RP241 Mercury and Selenium Relationships in Local Fisheries** N. Ares, Roger Williams Univ, Marine Biology; M. Soto, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnología, Roger Williams Univ, Marine Biology. Mercury (Hg) is a toxic environmental contaminant that negatively affects human health, and exposure occurs through the consumption of finfish. Selenium (Se) has the potential to directly or indirectly interact with Hg, and in the process, have a mitigating effect on Hg toxicity. Hg levels have been investigated in edible muscle filets of fish, e.g., summer flounder (*Paralichthys dentatus*), black sea bass (*Centropristis striata*), tautog (*Tautoga onitis*), and bluefish (*Pomatomus saltatrix*). Information on the Hg content of other tissues, e.g., brain and liver, however, is lacking. The brain is of particular concern because Hg is a neurotoxin, and the liver due to its role in detoxification. Moreover, the relationship between Hg and Se across these different tissues is largely unknown. This study examined Hg and Se concentrations among four estuarine finfish and their respective tissue-types. The specific objectives of this study were to: (1) examine Hg and Se bioaccumulation within three target tissues: muscle, brain, and liver, (2) examine the molar ratios of Se:Hg within tissues, and (3) compare Hg/Se differences among finfish as it relates to species-specific life-history characteristics. From June to August 2007-2010, target fish were collected from the Narragansett Bay estuary (RI, USA). Length (cm) was recorded for each fish, and total Hg was determined using automated atomic absorption spectroscopy. Total Se was measured using inductively coupled plasma mass spectroscopy. Molar ratios of Se:Hg were calculated to determine the protective quality of Se over Hg toxicity, with a ratio  $\geq 1$  showing a mitigating effect.

**RP242 Microbial Diversity and Mercury Concentrations Within a Subtropical Estuary** C.N. Ryan, Texas Tech Univ, Dept of Environmental Toxicology; S. Cox, Texas Tech Univ, Dept of Environmental Toxicology. Microbial communities serve an important role in the overall health and function of estuarine ecosystems. However, the majority of studies evaluating microbial community composition in these areas have been restricted to culture-dependant methods which invariably omit a substantial amount of species. This study evaluates the microbial community structure within a mangrove-dominated estuary ecosystem. Sediment samples ( $n=11$ ) were collected from the lagoon-side Round Island Park in Vero Beach, FL. Varying microhabitats within the park were sampled, including: red mangrove

(*Rhizophora mangle*) swamp, seagrass beds, sand bars, and a popular public wading area. DNA from each sample was extracted and analyzed via 454 pyrosequencing to identify microbial community composition. Samples were also analyzed for mercury content. A total of 877 bacterial genera were detected, with 35 genera shared between all 11 samples. Three genera (*Fusobacterium*, *Cytophaga*, and *Desulfosarcina*) contributed to differences within the 11 samples. The results of this study provide a greater understanding of the diversity of subtropical estuarine microbial communities.

**RP243 Mineralogy and Heavy Metal Mobility of Marine Sediment of Cape Town Harbour, South Africa** H.K. Okoro, Cape Peninsula Univ of Technology, Faculty of Applied Sciences; O.S. Fatoki, Cape Peninsula Univ of Technology, Cape Town, South Africa.; Dept of Chemistry; F. ADE-KOLA, Univ OF ILORIN, Chemistry; B. XIMBA, Cape Peninsula Univ of Technology., Chemistry; R. SNYMAN, Cape Peninsula Univ of Technology, Dept of Biodiversity and Conservation. Quantitative mineral analysis has been done by Powdered X-ray diffraction (PXRD) and Fourier Transform Infra-Red spectroscopy (FTIR) on marine sediment of Cape Town harbour. The heavy metal mobility was evaluated using ICP-MS and ICP-AES respectively. The results were statistically analysed using SAS 9 software (Cary, NC, USA). The posteriori ANOVA procedure was performed to determine the variation and significance variations in the dependent variables due to sites with a  $P \leq 0.05$  were considered significant. Some of the heavy metals analysed showed weak correlation of  $r < 0.7$  with the strong correlation coefficients between (Al and Si, Fe, Cu, Zn); (Si and Fe, Zn); (Fe and Zn); (Sn and Hg, Pb) (Hg and Pb) showing  $r > 0.7$  and significant at ( $P \leq 0.05$ ). Inversely lower correlation coefficients  $r < -0.3$  existed between (Hg and Al, Si, Fe, Zn) respectively. Scree plot was used to estimate the component variation in the heavy metals from the eigen values. The order of variation is  $Zn > Cd > Sn > Hg > Pb > Al > Si > Fe > Cu$  respectively. The enrichment factors of Sn, Pb, Zn, Fe, Cd, Al and Hg revealed anthropogenic inputs of these metals into the marine environment. The enrichment factor of Cu was less than 1 and this suggests that its presence was largely due to natural changes. The results are indications of the contributions of heavy metals contained in the runoffs from the domestic and urban drains, as well as the inflow storm water. Ship repair activities appeared to constitute major factor responsible for the higher metal contamination in the dockyards areas. Mineralogical analysis using FTIR and XRD confirmed the presence of quartz, pyrite, carrolite and calcite as the major constituents of the marine sediment  
Keywords: Organic matter, mineral analysis, XRD, FT-IR, marine, pollutants, correlation coefficients,

**RP244 Modeling Polyunsaturated Fatty Acids (PUFA) in Freshwater Fishes: A Kinetic Approach** J. Sawyer, Univ of Toronto, Geography; N. Gandhi, Univ of Toronto, Chemical Engineering; M. Diamond, Univ of Toronto, Geography, Chemical Engineering. Polyunsaturated fatty acids (PUFAs) are crucial for fish because they affect metabolic activity, growth rates and reproduction. PUFAs cannot be synthesized by organisms at rates sufficient to meet their basic biochemical requirements and therefore must be obtained largely through the diet, magnifying through the aquatic food web. We propose a generic, kinetic-based PUFA model to simultaneously examine the fate and transport of omega-6 (18:2n6, 20:4n6) and omega-3 (18:3n3, 20:5n3, 22:6n3) fatty acids in fish, emphasizing freshwater species. Our multi-compartmental model simplifies the physiologic reality of PUFA metabolism, considering the mechanisms of accumulation, egestion, biotransformation and oxidation. PUFAs should be examined independent of other fatty acids as they each have their own distinct characteristics and behaviors. Understanding and quantifying fish tissue fatty acid profiles are of great importance given that PUFA are suggested to play a role in lowering heart disease risks, moderating tissue inflammation, and the development of the nervous, reproductive, and photoreception systems in humans.

**RP245 Mountain Top Removal Coal Mining in WV: Integrated Field and Laboratory Studies Elucidating Biological Consequences of a Complex Contaminant Mixture** M. Arnold, T. Lindberg, Duke Univ, Nicholas School of the Environment ; Y. Liu, Duke Univ, Pratt School of Engineering; A. Watson, Duke Univ, Nicholas School of the Environment ; H. Hsu-Kim, Duke Univ, Pratt School of Engineering; D.E. Hinton, R. Di Giulio, Duke Univ, Nicholas School of the Environment. The Mud River and its tributaries in West Virginia receive effluent from mountain top removal and valley fill (MTR/VF) coal mining operations. These effluents typically have increased conductivity and concentrations of potentially toxic

metals and compounds that may impact organisms living in these receiving waters. The goal of this study is to determine the biological effects of MTR/VF stream contaminants and to identify the key drivers of observed toxicity using the fathead minnow model as a sensitive measure of environmental health with complementary field studies using the creek chub as a sentinel species. Water quality and sediment contamination data have been collected for the Mud River and several tributary streams. Selenium is an essential micronutrient that is beneficial at low concentrations but at higher concentrations can be toxic to organisms, including fish and invertebrates. Concentrations of selenium in the Mud River were above the USEPA chronic exposure water criterion of 5 ppb for 43 of 52 samples at sites along the Mud River. Fish were captured using electroshocking equipment from a site along the Mud River impacted by MTR coal mining. Several of the fish (*Lepomis* sp. hybrids) collected exhibited slight to severe jaw deformities suspected to be caused by selenium exposure while no deformed fish were collected from sites along the Left Fork of the Mud River that are not impacted by MTR/VF operations. Selenium concentrations in the collected fish will be determined using ICP-MS techniques. Histological examination of fish tissues collected from Mud River sites and control streams not impacted by MTR coal mining will be performed to determine if exposure to the mixed contaminants from coal mining has caused alterations in fish development. Adult fathead minnows will be exposed to selenium at levels comparable to those found at sites along the Mud River to identify toxic effects on offspring as well as genetic markers of oxidative stress in both adults and embryos.

**RP246 Multi-level Assessment of Sublethal Toxicity of Copper and Benzo[a]pyrene Toward Pale Chub** W. Kim, Korea Institute of Toxicology, Environmental toxicology Center, Korea Univ, Division of Environmental Science & Ecological Engineering; J. Lee, Korea Institute of Toxicology, Ecotoxicology lab; J. Kim, E. Lim, Korea Institute of Toxicology; S. Lee, Korea Institute of Toxicology, Ecotoxicology Laboratory; S. Lee, K. Choi, Seoul National Univ; J. Jung, Korea Univ. Multi-level biomarker responses (molecular/biochemical and histological/physiological levels) were studied to assess sublethal toxicity of copper (Cu) and benzo[a]pyrene (BaP) toward pale chub, *Zacco platypus*. No significant differences at the histological or physiological levels were observed among treatment groups, except for kidney tissue exposed to 20 µg Cu/L. However, various molecular/biochemical responses were observed in *Z. platypus*, and these primarily depended on exposure time. Upon Cu exposure, both DNA single-strand breaks (COMET) and metallothionein (MT) activity were significantly increased for 4 days, while there was no significant change after 14 days of exposure. BaP exposure induced a significant increase in COMET and 7-ethoxyresorufin-O-deethylase (EROD) activity at 4 and 14 days of exposure. Additionally, BaP induced acetylcholinesterase (AChE) activity at 14 days of exposure. Standardized scores of biomarker responses at molecular/biochemical levels were visualized using star plots and computed as the integrated biomarker response (IBR). The IBR values well correlated with the concentrations of Cu and BaP, and the correlations were better at 4 days of exposure ( $r^2 = 0.780$  and  $0.699$ , respectively) than 14 days ( $r^2 = 0.692$  and  $0.548$ , respectively). These results indicate that short-term exposure may work as well or even better for quantification of various biomarker responses at the molecular/biochemical levels when compared to long-term exposure.

**RP247 Multigenerational Exposure of *Heterandria formosa* to 17 $\alpha$ -ethinylestradiol: Organism and Population Level Effects Through Two Life Cycles** L.M. Carrier, P.L. Klerks, Univ OF LOUISIANA AT LAFAYETTE, Dept of Biology. The presence of endocrine disrupting compounds (EDCs) in the environment has gained major worldwide attention because of the possibility that EDCs may be affecting the reproductive health of wildlife populations and humans. The synthetic estrogen, 17 $\alpha$ -ethinylestradiol, is a potent endocrine modulator and is present at biologically active concentrations in aquatic ecosystems. To investigate impacts of EE2 on an aquatic environment, a 150-day in vivo study is examining the potential effects of the synthetic estrogen 17  $\alpha$ -ethinylestradiol (EE2) in the least killifish (*Heterandria formosa*). Newborn fish are being exposed to 1.0ng/L EE2, 5.0ng/L EE2, or a solvent control (100% ethanol) in a static-renewal system with replacement every 72 hours. Effects of EE2 on growth, sex ratio, survival rate, population growth rate, reproduction rate, and time to sexual maturity in the developing fish are being examined through two generations. Reduced growth, female-biased sex ratios, decreased survival, diminished populations, abated reproduction, and a longer time to sexual



maturity are expected for the EE2-exposed fish. Results of the ongoing experiment will be presented at the meeting.

**RP248 Parasites and Pollution: A Study of Selenium Uptake in Nematode Infected Rainbow Trout (*Oncorhynchus mykiss*)** O. Hursky, M. Pietrock, Univ of Saskatchewan, Toxicology Centre. Given that parasites demonstrate different sensitivity to contaminants and environmental stress there is an increasing interest in using parasites as biological or ecological indicators of their fish host life conditions as well as bioindicators of heavy metal pollution of aquatic ecosystem. The aim of this study was to investigate (1) whether there is a transfer of selenium from intestinal contents of rainbow trout (*Oncorhynchus mykiss*) to nematodes (*Raphidascaris acus*), (2) whether parasites bioconcentrate more selenium than their host and (3) to examine if the combined effect of parasitic infection and Se exposure has an effect on fish health (as determined by Fulton's condition factor, Hepatosomatic Index (HSI), Gross Energy (GE) content and levels of catalase and GST). Rainbow trout were infected with larval stages of nematodes and subsequently exposed to dietary Se for 70 days (4.5 mg Se /kg food w.w.) Selenium concentration of trout muscle tissue and intestinal parasites were determined using ICP-MS. The results indicate that comparing to muscle tissue of their host, *R. acus* accumulate lower levels of Se, therefore is not a good bioindicator of Se accumulation in ecosystem. However, *R. acus* do have an impact on fish health: combined with Se exposure parasitic infection decreases GE content of the fish and alters enzyme levels.

**RP249 Pollutant Sensitivity of the Green Floater (*Lasmigona subviridis*), a Freshwater Mussel of Conservation Concern in Mid-Atlantic River Basins, USA** D.K. Hardesty, J.M. Besser, USGS; C.G. Ingersoll, USGS, Columbia Environmental Research Center; N. Wang, USGS; T. Augspurger, R. Mair, USFWS. The green floater (*Lasmigona subviridis*) is a species of concern in North Carolina, Virginia, and other mid-Atlantic states due to declining numbers in the wild. The species has a unique life cycle in that females produce transformed juveniles within their gills (not requiring a parasitic stage on a fish host). The pollutant sensitivity of the species is of interest in determining the extent to which water quality may be a limiting factor in their survival and recovery. Also, green floaters might serve as a good organism for use in laboratory toxicity testing, given that no host fish is needed for transformation of juveniles from the glochidia life stage. We conducted 96-hour exposures of < 5 day-old juvenile green floaters to copper, zinc, and chlorine in separate exposures. Control survival was 100% in all tests. The EC50s (immobilization) for copper (26 µg/L at a hardness of 162 mg/L as CaCO<sub>3</sub>), zinc (472 µg/L at a hardness of 50 mg/L), and chlorine (135 µg/L) indicates green floaters were not particularly acutely sensitive compared to other mussel species. Interestingly, there was considerable "recovery" of effected organisms in clean water for 24 hours following the initial 96-hour exposures to toxicants. This type of a recovery has been observed in older mussels (e.g., 2-month-old), less so for newly released juveniles of all others species which use the obligate fish host for transformation of juveniles.

**RP250 Population Level Assessment of the Concomitant Impacts of Temperature and Lead Using *Daphnia magna*** N. Melby, US Army ERDC and Mississippi State Univ; J.D. Laird, US Army Engineer Research & Development Center, SpecPro Incorporated, US Army Engineer Research & Development Center, Environmental Laboratory; D. Meeks, A.J. Kennedy, A. Casper, US Army Engineer Research and Development Center; S. Brasfield, US Army Engineer Research and Development Center, Environmental Laboratory. The focus of this study is to determine the potential for interactive effects of chemical and nonchemical stressors, primarily lead and temperature related to climate change scenarios on military installations using the freshwater cladoceran, *Daphnia magna*. This approach may provide information as to whether predicted changes could affect contaminant management on military installations. Lead was chosen as the chemical stressor because of its importance related to small arms firing ranges on DoD military installations where lead ammunition is used for military training exercises. These metals can leach into the soils which then have the potential to run off into aquatic environments where toxicity effects on the organisms with climate change have yet to be investigated over time in a population model. Endpoints measured for this species include growth, reproduction, and population demographics through numerous 21-day exposures in environmental chambers at varying temperatures and also with lead (II) chloride (PbCl<sub>2</sub>). Predictions are that the chronic toxicity of metals will increase due

to the shift in climate change as multiple stressors on *D. magna*. Temperature only exposures at 20, 23, and 26°C elicited a small negative response in weight and size as well as reproductive effects on the *Daphnia* over 21d. High temperatures result in earlier reproduction, increase in the number of broods per individual, however a decrease in the number of neonates per brood. When the effect of treatment temperature is compared on an individual basis, the 23°C and 26°C organisms begin producing broods two days earlier and produced two more broods than the organisms exposed at 20°C. However, when the mean number of neonates per brood is calculated, a linear negative relationship with temperature resulted. *Daphnia* exposed at higher temperatures have the capability to reproduce earlier but are exposed to the chemical for a longer period of time; therefore, they may be more sensitive due to increased metabolism. Energy is being shunted between maintenance function, growth and/or reproduction for each treatment.

**RP251 Potential Effects of Life History Evolution on Ecological Risk Assessment** M. Kamo, Advanced Industrial Science and Technology, Advanced Industrial Science and Technology, Research Institute of Science for Safety and Sustainability; T.I. Hayashi, National Institute for Environmental Studies; T. Akita, Graduate Univ for Advanced Studies. We theoretically investigated how the sensitivity of organisms to the toxicity of chemicals varies depending on their life history traits. We considered a simple resource allocation model combined with a logistic growth equation. We assumed that organisms allocated the resources to (1) reproduction, (2) maintenance of their life and (3) mitigation of the adverse effects of chemicals with certain ratio. The ratio was considered to be the life history. We assumed that the ratio was subjected to evolution, and the evolutionary stable strategy (ESS) was investigated. We assumed two situations for evolution to occur. In one situation evolution occurred in the sparse population to mimic a laboratory condition, and in the other situation evolution occurred in crowded population to mimic a natural condition. We first investigated when the evolution occurred in the absences of chemicals. We found that an organism adapted in sparse population spend more resources on reproduction than that adapted in crowded population, and hence became r-strategists. When the effects of chemicals were measured by a toxicity reducing the population growth rate by 10% (EC10), the r-strategists were less sensitive to toxicity of chemicals. The result suggests that use of laboratory-reared organism in toxicity tests leads to the underestimation of ecological risk. When the adaptation occurred in the presence of chemicals, a strategy allocating for the mitigation of the toxicity became ESS. An amount of resource allocation for the mitigation in the evolutionary stable strategists depended on the shape of a function determining the relationship between the amount of resource and the effect of mitigation. In some cases, evolutionary bistability appeared and hence the organism became either chemical tolerant type or non-tolerant type depending on initial conditions of the evolution. Our results explain the diversity in chemical sensitivities within biological species.

**RP252 Recovery of Photosynthesis and Growth Rate in Green, Blue-Green, and Diatom Algae After Exposure to Atrazine** R. Brain, Syngenta Crop Protection, LLC., Dept of Environmental Risk Characterization; A. Hosmer, Syngenta Crop Protection, LLC.; S.B. Wall, Syngenta Crop Protection, LLC., Ecological Sciences. The effects of atrazine, a photosystem II (PSII) inhibiting herbicide belonging to the s-triazine family, on primary producers are reversible. In laboratory studies, recovery from atrazine exposure concentrations high enough to inhibit PS II has been documented in a variety of aquatic plants belonging to taxonomically diverse growth forms including floating macrophytes (*Lemna gibba*), submerged rooted macrophytes (*Potamogeton perfoliatus* and *Myriophyllum spicatum*), and green algae (*Scenedesmus vacuolatus*). In green algae, photosynthesis, as measured using pulse amplitude modulation (PAM) fluorometry, recovers immediately and growth recovers within hours. In order to expand upon the existing compliment of species and provide comparable recovery data, studies with green (*Pseudokirchneriella subcapitata*), blue-green (*Anabaena flos-aquae*), and diatom (*Navicula pelliculosa*) algae were conducted to evaluate the effects of atrazine on photosynthesis (measured using PAM fluorometry) and growth rate. Algae were grown for 24 to 48 hrs, then exposed to atrazine for 48 hrs and evaluated for recovery for a further 48hrs after removal from exposure. Collectively these studies further corroborate the conclusion that primary producers recover quickly from exposure to atrazine at concentrations high enough to inhibit PSII, where recovery of photosynthesis begins immediately and recovery of growth rate begins within hours. Species



specific differences in sensitivity were observed, though recovery kinetics were largely comparable and consistent.

**RP253 Recovery of Wild Small Bodied Fish Populations After a Multi-year Exposure to a Synthetic Estrogen** B. Park, Dept of Fisheries & Oceans Canada, Dept of Fisheries and Oceans, Freshwater Institute, Dept of Fisheries and Oceans; V. Palace, Freshwater Institute, Dept of Fisheries and Oceans Canada; K. Wautier, P. Blanchfield, Freshwater Institute, Dept of Fisheries and Oceans; K. Kidd, Canadian Rivers Institute, Univ of New Brunswick, Biology. Estrogenic contaminants are routinely detected in surface waters, and can disrupt reproductive endocrine system function in aquatic organisms. To address potential impacts on wild fish populations, we added the synthetic estrogen 17 alpha-ethynylestradiol (EE2) to an experimental lake (L.260) from spring to fall of 2001 to 2003, and monitored various physiological and population parameters from the EE2-treated lake and two reference lakes. Previously we reported that EE2-exposed fish exhibited vitellogenin induction in males, inhibited gonad development in both sexes, testicular oocytes, and histopathological changes in liver and kidney. The fathead minnow (*Pimephales promelas*) population in the treated lake collapsed due to lack of recruitment, whereas these effects were absent in reference fish populations. To assess potential recovery of fish in L.260, we continued to monitor these populations after cessation of the EE2 additions for vitellogenin, histology and population endpoints. Fathead minnow and pearl dace (*Margariscus margarita*) were collected in spring and/or fall from 2004 to 2010. Preliminary results indicate that reproductive abnormalities were present in both species two years post-exposure in L.260, but were absent in subsequent years. There was no difference among lakes in vitellogenin levels in male fathead minnows caught in the fall of 2010; analyses of samples for other dates and for pearl dace are ongoing. Catch-per-unit-effort (CPUE) of adult fathead minnow returned to pre-experiment levels by the spring of 2007 (4 years post addition). These results indicate that, given time, fathead minnow and pearl dace recovered across all levels of biological organization from the whole lake additions of EE2.

**RP254 Relationships Between PCDDs, PCDFs, Dioxin-like PCBs, and Other Compounds of Concern in Fish from the Saginaw Bay Watershed (Lake Huron, MI, USA)** R. Holem, J. Newsted, Cardno Entrix; S. Roark, CH2M Hill, Ecotoxicology & Risk Assessment; J. Matousek, Cardno Entrix; D. Tazelaar, Michigan State Univ, Animal Science, Cardno Entrix; J.P. Giesy, Univ of Saskatchewan, Biomedical Veterinary Sciences & Toxicology Centre, Michigan State Univ, Dept of Animal Science; D. Kay, Cardno Entrix. Previous investigations have reported elevated concentrations of dioxin-like compounds in sediments, floodplain soils, and tissues of fish collected from the Saginaw Bay watershed. In 2007 fish were collected from the Tittabawassee and Saginaw Rivers and Saginaw Bay of Lake Huron to provide data for human health risk assessment and further characterize contaminant levels in fish fillet tissues. In 2008, fish were collected from the upper Tittabawassee, Pine, Shiawassee, Flint, and Cass Rivers, to provide comparison contaminant data for fish from tributaries of the Tittabawassee and Saginaw Rivers. Concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated-biphenyls (dl-PCBs), mercury, arsenic, and a suite of other compounds were measured in nearly 500 fish of the fish collected in 2007 and 2008. Species included carp, channel catfish, black crappie, freshwater drum, largemouth and smallmouth bass, northern pike, yellow perch, sunfishes, and the migratory species walleye, white bass, and white suckers. Average concentrations of  $\Sigma$ PCDDs and  $\Sigma$ PCDFs ranged from 1–18 and 2–60 pg/g wet weight (ww), respectively, while average  $\Sigma$ dl-PCBs ranged from 15–150 ng/g ww. Concentrations of  $\Sigma$ PCDDs,  $\Sigma$ PCDFs,  $\Sigma$ dl-PCBs, and tetrachlorodibenzo-*p*-dioxin equivalents (TEQ) were greatest in carp and channel catfish and generally least in freshwater drum and sunfish species. In general, furan concentrations were greatest in fish from the Tittabawassee River while dl-PCBs were greatest in Saginaw River fish. Mercury, 4,4'-DDE, and arsenic were detected in nearly all fish analyzed. Principal components analysis was conducted to examine differences in contaminant patterns between predatory and benthic-feeding species and between migratory and resident species. In addition, contaminant patterns were examined between the different locations with specific focus on differences between the Tittabawassee and Saginaw Rivers and their tributaries.

**RP255 Salinity Acclimation Modulates Copper Toxicity in the Sheepshead Minnow, *Cyprinodon variegatus*** J.A. Adeyemi, Univ of Louisiana at

Lafayette, Dept of Biology; P.L. Klerks, Univ of Louisiana at Lafayette, Dept of Biology. The sheepshead minnow, *Cyprinodon variegatus* is a euryhaline fish that is able to withstand a very wide range of salinities. In two experiments we examined the effects of salinity acclimation on copper toxicity in sheepshead minnows. In the first experiment, fish were pre-exposed to 2.5, 10.5 or 18.5 ppt seawater for 14 days, after which they were exposed to a fixed free cupric ion level (16.6  $\mu$ M Cu<sup>2+</sup>). Survival during the 14-day salinity acclimation was high in all the groups. However, fish from these groups responded differently during Cu challenge. Fish pre-exposed to 2.5 ppt salinity were more sensitive (about 70% mortality) compared to fish pre-exposed to 10.5 or 18.5 ppt seawater. In the second experiment, fish were exposed to 14.6  $\mu$ M Cu<sup>2+</sup> for 6 hours after the 14-days salinity acclimation. Fish were subsampled across groups for determination of whole-body Cu, liver lipid peroxidation, liver catalase activity and liver glucose levels before (T0) and after (T6) copper exposure. At T0, there were no differences across the groups for most of the endpoints measured in this study, except for liver glucose level that was significantly higher in 2.5 ppt acclimated fish compared to the other groups. At T6 whole-body Cu and liver lipid peroxidation differed significantly among the groups, but there was no difference in liver glucose level or catalase activity. This study showed that while these fish were able to acclimate to substantial salinity changes, this adjustment came with a large metabolic cost that resulted in reduced tolerance during subsequent copper exposure.

**RP256 Scaling Up Bioassays for Toxicogenomics: Impacts on *Daphnia magna* Sensitivity to Metals** A.J. Kennedy, US Army Engineer Research and Development Center; J.D. Laird, US Army Engineer Research & Development Center, SpecPro Incorporated, US Army Engineer Research & Development Center, Environmental Laboratory; B. Meeks, US Army Engineer Research and Development Center; K.A. Gust, US Army, Engineer Research and Development Center, Environmental Laboratory, US Army, Engineer Research and Development Center, US Army Engineer Research & Development Center, ERDC-EL-EP-P; M.S. Wilbanks, US Army Engineer Research & Development Center; R. Nisbet, University of California, Santa Barbara; E.J. Perkins, US Army, Engineer Research & Development Center, Environmental Laboratory, US Army Engineering Research & Development, Environmental Processes & Effect Division. As toxicogenomics and dynamic energy budget (DEB) determinations become integrated with traditional ecotoxicological endpoints and environmental risk assessments, modifications to standard bioassays will become necessary. Standard acute or chronic bioassays employing the aquatic receptor *Daphnia magna* do not currently supply adequate tissue mass for conventional microarray-based gene expression assays. Therefore, standard bioassays must be scaled up in terms of number of *D. magna* and chamber size. Further, DEB analyses require accurate determinations of nutritional carbon, which is complicated by the heterogeneous standard feeding ration (i.e., algae and yeast-cerophyl-trout chow, or YCT). To understand how scaled up test methods for toxicogenomics and DEB analysis may impact response to metals, the standard 21-day *D. magna* chronic toxicity test method (one organism per 50 ml exposure vessel) was compared to a modified method (20 organisms per 1000 ml exposure vessels). The model chemicals were copper (Cu) and lead (Pb). Bioassays were conducted at 24 °C and received algae-only feeding rations, denoted 1X (standard ration) and 2X (double the standard ration). *Daphnia magna* were significantly more sensitive to both metals in the modified method relative to the standard method. Removing YCT from the standard feeding ration (1X algae-only) significantly reduced reproduction; however doubling the algae ration (2X) mitigated the effect. Regardless of the test method or metal, significant reductions in survival occurred at first molt and first brood, suggesting these are important stages to consider in genomics and DEB. Using the modified method, *D. magna* survival in Pb exposures was slightly but significantly more sensitive when provided 1X feeding ration (LC50 = 68 [63–73] ppb) compared to the 2X ration (LC50 = 79 [74–84] ppb). While the 2X feeding ration generally resulted in statistically increased growth and reproduction across all Pb treatments, the 1X feeding ration resulted in a significant sublethal impact at a lower Pb concentration. Findings suggest that when the objective is to relate toxicogenomics to standard ecotoxicological effects or DEB analysis, all endpoints should be generated from the modified method used to generate tissue for toxicogenomics since extrapolation to the standard method may not be directly relatable in terms of response to chemicals.

**RP257 Selection of Stream Insect Larvae for Indicating Anthropogenic Impact** J. Lake, USEPA, Atlantic Ecology Division; J. Serbst, A. Kuhn, USEPA. This study examined the total mercury concentrations, [Hg], and  $\delta^{15}\text{N}$  values in macro-invertebrates collected from 35 stream sites in Rhode Island, USA, to determine the organism groups most suitable for use as indicators of anthropogenic impact. Site selection was designed to cover a wide geographical range and to encompass relatively pristine locations in the western part of the state and others from more highly developed and populated areas near the city of Providence. Sites were sampled on an irregular basis over a two-year time period. Samples were collected by dip and kick nets and consisted largely of insect larvae. The distributions of larval insects varied among sites. The most common larval organism groups, found at 30 and 28 sites, respectively, were: hellgrammites, a group that is a combination of dobsonflies and fishflies, and damner dragonflies. The larvae of both of these groups are engulfer-predators. Across sites, relationships between [Hg] and  $\delta^{15}\text{N}$  varied among the organism groups. Engulfer-predators showed significant negative relationships between [Hg] and  $\delta^{15}\text{N}$  values, except for skimmer dragonflies, which did not show a significant relationship. The  $\delta^{15}\text{N}$  value of organisms has been found to reflect the level of nitrogen nutrients in fresh water sites which often results from human wastewater inputs. Therefore, the negative relationships shown for most of the engulfer-predators may reflect anthropogenic impacts across sites. However, pH varies among sites and explains 31% of the variance in [Hg] for hellgrammites. When pH was combined with the  $\delta^{15}\text{N}$  data for this organism group in a stepwise regression, 43% of the variance in [Hg] was explained. Overall, the frequency of occurrence and the strength of the relationship between [Hg] and  $\delta^{15}\text{N}$  for hellgrammites appear to support their potential use as an indicator of the magnitude of Hg contamination and anthropogenic impact in streams. At sites where hellgrammites are not found, damner dragonflies could be substituted because the relationships between [Hg] and  $\delta^{15}\text{N}$  were not significantly different between these two groups.

**RP258 Serum SDH Activity as a Biologically Significant Biomarker of Liver Integrity in Fish** D. Webb, Curtin Univ, Environment and Agriculture; M. Gagnon, Curtin Univ, Dept of Environment and Agriculture, Curtin Univ (Bentley Campus), Dept of Environment and Agriculture, Curtin Univ, Environment and Agriculture. In order to assess the usefulness of serum dehydrogenase (SDH) activity as a biomarker of liver damage in fish, black bream (*Acanthopagrus butcheri*) were injected with a model hepatotoxicant, pentachlorophenol (PCP) sodium salt. A suite of biomarkers were measured over time, i.e., serum SDH activity, liver somatic index (LSI) and hepatocellular hyperplasia. At 3 days post-injection, serum SDH activity was significantly elevated in the fish group i.p. injected with 30 mg PCP/kg fish, relative to the negative control group. While SDH activity returned to control levels 6 days post-injection, the LSI increased significantly relative to control levels at this time. Histological damage, measured by hyperplasia, was not detected until day 9 post-injection. This study demonstrates that in black bream exposed to hepatotoxicants, changes in serum SDH activity are detectable before physiological or histological changes became evident. Serum SDH activity can therefore be used as a non-lethal, biologically meaningful early warning indicator of liver integrity.

**RP259 Studies on the Recovery of a River Ecosystem in Northern Ontario Previously Exposed to Pulp Mill Effluent** T. Arciszewski, Univ of New Brunswick, 5000+; K. Munkittrick, Univ of New Brunswick, Canadian Rivers Institute, Univ of New Brunswick, Dept of Biology. Studies on the impacts of pulp mills have been conducted at many sites in Canada since the late 1980s. Many of these studies have shown depressed circulating steroids and reduced gonad size. Temporary shutdowns and process improvements were often associated with moderate recovery of fish sentinels. In Jackfish Bay, Ontario, short-term shutdowns often elicited recovery of endpoints with high turnover, like mixed function oxygenases (MFOs) in the liver. A shutdown lasting 18 months also showed some reversal in the effects on fish, but not all impacts disappeared. Since 2000, the Canadian pulp and paper industry has contracted by approximately 50 %. With the permanent closure of many of these facilities comes the opportunity to more thoroughly study the recovery of ecosystems previously exposed to pulp mill effluents. At one kraft mill in Smooth Rock Falls, Ontario, Canada (which was closed permanently in July 2006), there was extensive work done while the mill was operating including collections by Environment Canada (Moose River Project; 1991-1997) and four cycles of Environmental Effects

Monitoring (EEM; 1995, 1998, 2002, 2005). The existing dataset includes performance data (body condition, growth rate, age, gonadosomatic index (GSI), liver somatic index (LSI), liver enzymes (mixed function oxygenases; MFO), circulating sex steroids, and fecundity) from white sucker (*Catostomus commersoni*) collected in 1993-1998, 2002, and 2005. During the operation of the mill, from 1993 to 2005, strong and recurrent effects were found in female gonad size; significant ( $p < 0.05$ ) effects on GSI of female fish ranged from -5 to -38% compared to an upstream reference site. Given the impacts of the previous discharge of now-closed mills on fish reproduction, we have initiated studies to examine the recovery of the Mattagami River which received the pulp mill effluent from the Smooth Rock Falls mill. To better understand recovery processes and their potential rate, this study will use the same sentinel fish species (white sucker), study locations, and methodology to compare the operational (1993-2005) and post-operational (2011) data sets.

**RP260 Subchronic Exposure to PFOA and PFOS in Zacco platypus, an Indigenous Korea Freshwater Fish** S. Kim, School of Public Health, Seoul National Univ, Environmental Health, Seoul National Univ, Graduate School of Public Health; S. Lee, Seoul National Univ, #306, Graduate School of Public Health; W. Kim, Korea Institute of Toxicology; C. Kim, A. Kim, Seoul National Univ, #306, Graduate School of Public Health; J. Kim, E. Lim, J. Lee, Korea Institute of Toxicology; J. Kim, Korea Research Institute of Bioscience and Biotechnology; S. Lee, Korea Institute of Toxicology; K. Choi, Seoul National Univ, #306, Graduate School of Public Health. Pale chub (*Zacco platypus*) is an indigenous freshwater fish which distributes widely in East Asia including Korea. This fish has been suggested for potential indicator species, however, information on molecular level biomarkers of *Z. platypus* is limited, and its application to ecosystem risk assessment is difficult. We selected several molecular level biomarkers and utilized the molecular biomarkers to assess toxicity mechanisms of PFOA and PFOS for 14-days exposure. Comet assay, acetylcholine esterase (AChE) assay, oxidative stress-related mRNA expression assay, oxidative stress-induced enzyme assay, and lipid peroxidation assay were conducted using several organs of *Z. platypus*. The results of comet assay and acetylcholine esterase assay suggest that PFOA can induce DNA damage and can affect neurotransmission in *Z. platypus* after 14 d exposure at 0.5 and 50 mg/L. Exposure to PFOA lead to increased activity of SOD in muscle, and lipid peroxidation in liver. However, exposure to PFOS did not show such protein level changes in liver. Histological level changes in gill, liver, kidney and gonads were not observed after PFOS exposure. Our study showed that PFOA exposure could lead to damages in DNA and neurotransmission, and oxidative stress may be in part responsible for such damages in *Z. platypus*. Consequences of long term exposure to PFOA deserve further investigation.

**RP261 Survival and Growth of Estuarine Fish in Response to Varied Exposures to Chemically Enhanced Dispersed Oil from the Deepwater Horizon Oil Spill** R.A. Brewton, Gulf Coast Research Lab, Univ of Southern Mississippi, Coastal Sciences; J. Griffith, USM, faculty; R. Fulford, Gulf Coast Research Lab, Univ of Southern Mississippi, Coastal Sciences. To assess potential impacts of the 2010 *Deepwater Horizon* oil spill on coastal habitats, we are examining the effects on individual fish in laboratory exposures. Spotted seatrout (*Cynoscion nebulosus*) are considered a good indicator of estuarine health because they occupy a mid-trophic level, are estuarine residents and show great plasticity in their growth due to environmental conditions. Laboratory assays of survival and growth of spotted seatrout as a function of dispersed oil concentration will be conducted at Gulf Coast Research Laboratory (GRCL) Toxicology Research Center using spotted seatrout from the GCRL hatchery. Weathered oil will be produced for experiments by mixing crude oil obtained from British Petroleum with seawater for 24 hours creating a water accommodated fraction (WAF); chemically enhanced weathered oil (CEWAF) will be produced with the same methods using both crude oil and dispersant (Corexit 9500). Juvenile fish (N=144 individuals) will be exposed to either CEWAF, WAF or dispersant alone for 96 hours in a spiked exposure. There will also be a control treatment that receives nothing. At the conclusion of the exposure, individuals will be removed, weighed and measured and morphometric indices will be used to evaluate fish health. 2 individuals from each treatment will be immediately sacrificed by cervical dislocation. The surviving fish will be retained and grown for 6 weeks, and then sacrificed to evaluate if the effect of exposure to CEWAF, WAF or dispersant has on the growth response of the fish. To confirm exposure of the fish to hydrocarbons, biomarker stress responses to



hydrocarbons will be evaluated within these fish; such as Cytochrome P450-1A levels. A second spiked exposure will be done (4 tanks per treatment, 12 individuals per tank) to compare the effect time exposed to CEWAF has on growth with exposure times of  $t = 12, 48, 72$  and  $96$  hours. 2 fish from each treatment will be sacrificed at the conclusion of the exposure period, and the remaining individuals will be grown for 6 weeks, and then sacrificed. The results obtained from the laboratory assays will be compared to field growth and health data to test for habitat specific correlations between short-term stress and medium-term effects.

**RP262 Test of Significant Toxicity: A Better Statistical Application for Assessing Effluent Toxicity** D. Denton, USEPA Region 9, c/o SWRCB; J. Diamond, L. Zheng, Tetra Tech, Inc. The US Environmental Protection Agency (USEPA) and state agencies implement the Clean Water Act, in part, by evaluating the toxicity of effluent and surface water samples. A common goal for both regulatory authorities and permittees is confidence in an individual test result (e.g., NOEC, pass/fail, EC25), which is used to make regulatory decisions, such as reasonable potential determinations, permit compliance, and watershed assessments. This paper discusses an additional statistical approach (Test of Significant Toxicity [TST]), based on bioequivalence hypothesis testing, or more appropriately, test of non-inferiority, which examines whether there is a non-toxic effect at a single concentration of concern as compared to a control. Unlike the traditional hypothesis testing approach in whole effluent toxicity (WET) testing, TST is designed to explicitly incorporate both alpha and beta error rates at levels of toxicity that are unacceptable and acceptable, given routine laboratory test performance for a given test method. Regulatory management decisions are used to identify unacceptable toxicity levels for acute and chronic tests and the null hypothesis is constructed such that test power is associated with the ability to correctly declare a truly non-toxic sample as acceptable. This approach provides a positive incentive to generate high quality WET data to make informed decisions regarding regulatory decisions. We illustrate how alpha and beta error rates were established for specific test method designs, and tests the TST approach using both simulation analyses and actual WET data. In general, those WET test endpoints having higher routine (e.g., 50<sup>th</sup> percentile) within-test control variation on average, have higher method-specific alpha values (Type I error rate) so as to maintain a desired Type II error rate. We delineate the technical underpinnings of this approach and demonstrates the benefits to both regulatory authorities and permitted entities.

**RP263 The Effect of Ethynilestradiol on Gonopodium Morphology and Gonadal Histology of *Cnesterodon decemmaculatus* (Pisces, Poeciliidae)** B.J. Young, Laboratorio de Transformación de Residuos, IMYZA, INTA. ; G.C. Lopez, Laboratorio de Ictiofisiología y Acuicultura, IIB-INTECH (CONICET-UNSAM); D. Crespo, Laboratorio de Transformación de Residuos, IMYZA, INTA. ; G.M. Somoza, Laboratorio de Ictiofisiología y Acuicultura, IIB-INTECH (CONICET-UNSAM); P. Carriquiriborde, Conicet, Centro de Investigaciones del Medio Ambiente, Facultad de Ciencias Exactas, Universidad Nacional de la Plata, Química, Centro de Investigaciones del Medio Ambiente – UNLP, CONICET, Facultad de Ciencias Exactas, UNLP. Public and scientific concern about EDCs has claimed for the understanding the mechanism underlying adverse effects and the development of ecotoxicological tools for assessing potential environmental impacts. Ethynilestradiol (EE2) is the main estrogenic component of the combined oral contraceptive pills, and its presence in surface waters has been reported worldwide. Holarctic Poeciliid fish has demonstrated to be valuable indicators of exposure to endocrine-modulating substances, because they exhibit a hormone-dependent sexual dimorphism. In the present study, alterations at the morphology of the gonopodium and the gonadal histology of the Neotropical Poeciliid, *Cnesterodon decemmaculatus*, in response to the EE2 were assessed under laboratory conditions. Two experiments were conducted exposing adult fish to environmentally and pharmacologically relevant waterborne concentration of EE2, ranging from 25 to 250 ng/L during a 90 days period. The description of the normal morphology of the gonopodium helped to build a gonopodial index for characterizing developmental stages in the studied species. Nevertheless, gonopodium morphology was not sensitive to environmental EE2 exposure, showing no response after 84 d of exposure. However, testicular histology showed primary oocytes in the outer sector since day 54 in fish exposed to 250 ng EE2/L. The number of oocytes increased and started to invade the central zone of the testis by day 84. Moreover a parenchymatization of the seminal tube lumen was also observed. The study demonstrated the induction of ovotestis ("intersex") by

EE2 in *C. decemmaculatus*, encouraging the utilization of this local fish as test species for evaluating the impact of EDCs.

**RP264 The Effects of Copper Amended Marine Sediments on an Estuarine Amphipod and Marine Polychaete During Chronic Bioassays** A.K. Gerke, K.E. Gaertner, ABC Laboratories, Ecotoxicology; J. Gorsuch, Copper Development Association. With dispersive forms of copper in stormwaters reaching coastal surface waters and the use of copper as an active ingredient in anti-fouling paints, some marine sediments are accumulating copper. Thus, concerns over the potential effects of copper to benthic saltwater organisms have increased during the last decade. However, based on the intricacies of sediment toxicity and the complex behavior of metals in the environment, very few studies have managed to address this issue adequately. In most published studies, the effects of oxidization during the spiking procedure, binding to ligands, as well as sediment to porewater partitioning of metals have greatly been ignored or underestimated. Considering partitioning alone, toxicity has typically been overestimated due to combined sediment and porewater toxicity. Additionally, a majority of studies that have been based on equilibrated sediments neglected to investigate sediment properties which potentially influence toxicity. Recent work has established factors such as organic matter and particle size to affect benthic organisms in the presence of copper. During the past decade efforts have been made to predict toxicity based on ratios of simultaneously extracted metals (SEM) and acid-volatile sulfides (AVS), and organic matter measured as total organic carbon (TOC) or organic matter lost on ignition (LOI). The use of SEM and AVS to predict toxicity does have limitations. To investigate the toxicity of copper in equilibrated marine sediments, we conducted two 28-day chronic copper sediment exposures using two benthic species with different foraging behavior: the estuarine benthic amphipod, *Leptocheirus plumulosus* (ASTM E-1367), and the burrowing marine polychaete, *Neanthes arenaceodentata* (ASTM E-1611). Based on analyzed porewater and sediment concentrations of copper, preliminary results indicate that copper in equilibrated marine sediments is less toxic to these organisms than had been identified for similar species. A full analysis of these findings will be presented.

**RP265 The Joint Effects of Sulfonamides and Their Potentiator on *P. phosphoreum*: Differences Between the Acute and Chronic Mixture Toxicity Mechanisms** Z. Lin, D. Yin, Tongji Univ, College of Environmental Science and Engineering; X. Zou, Tongji Univ, College of Environmental Science and Engineering, Tongji Univ. Organisms are typically exposed to mixtures of chemicals over long periods of time; thus, chronic mixture toxicity analysis is the best way to perform risk assessment in regards to organisms. However, most studies focus on the acute mixture toxicity. To investigate the difference between chronic mixture toxicity and acute mixture toxicity, *Photobacterium phosphoreum* were exposed to chronic (24 h exposure) and acute (15 min exposure) toxicity of single sulfonamides (SAs) and their potentiator (trimethoprim, TMP), both individually and as mixtures. A comparison of chronic vs. acute mixture toxicity revealed the presence of an interesting phenomenon, that is, that the joint effects vary with the duration of exposure; the acute mixture toxicity was antagonistic, whereas the chronic mixture toxicity was synergistic. This phenomenon was due to the presence of two points of dissimilarity between the acute and chronic mixture toxicity mechanism: (1) the receptor protein of SAs in acute toxicity was Luc, while in chronic toxicity it was Dhps, and (2) there is a difference between actual concentration of binding-Luc in acute toxicity and individual binding-Dhps in chronic toxicity. This deep insight into the difference between chronic and acute mixture toxicity will benefit environmental science, medical science, and other disciplines. The existence of these differences poses a challenge for the assessment of routine combinations in medicine, risk assessment, and mixture pollutant control, in which, previously, only a synergistic effect has been observed between sulfonamides and their potentiator.

**RP266 The Observation of Epibionts on Laboratory Cultures of *Hyalella azteca* and its Implications for Organism Performance in Toxicity Tests** T.W. Valenti, National Research Council; US Environmental Protection Agency, National Research Council; S. Landers, Troy Univ, Dept of Biological and Environmental Sciences; T. Highland, R. Hockett, US Environmental Protection Agency, Mid-Continent Ecology Division; T.J. Norberg-King, US Environmental Protection Agency, ORD, NHEERL, Mid-Continent Ecology Division, USEPA, Mid-Continent Ecology



Division; S. Peterson, EMR, biologist; D.R. Mount, US Environmental Protection Agency, ORD. Epibionts are organisms that live on the surface of other living organisms. Several studies have identified epibionts on wild caught marine and freshwater crustaceans and they are often regarded as harmless to host organisms at low densities. However, epibionts may heavily infect laboratory populations of host organisms because cultures are typically carried out in semi-closed systems with high densities of potential host organisms. Under these scenarios, it is possible that the health and fitness of infected host organisms may be adversely affected. At our laboratory, the observation of high densities of epibionts on *Hyalella azteca* (at times > 500, individual) showed some correspondence with reduced performance in control treatments during standardized 10 d sediment toxicity tests. Interestingly, high rates of infestation did not appear to reduce survival or fecundity of adult breeding cultures, but rather were only related to lower rates of growth for 7-8 d old organisms during 10-d experiments. Ongoing efforts are targeted at not only identifying this species of epibiont, but also determining if these epibionts are the direct cause of reduced performance or whether the epibiont infection is a secondary result of another stressor. The objectives of this presentation are to: 1) inform scientists involved with culturing and testing of *Hyalella* about the potential presences of epibionts, 2) review the physical characteristics of the epibionts observed on our culture organisms, 3) describe previous efforts to eradicate or reduce epibionts infection in our cultures, and 4) summarize ongoing efforts to elucidate relationships between epibionts and *Hyalella* performance during 10 d sediment toxicity tests. This abstract does not necessarily reflect USEPA policy.

**RP267 Three Years of Continued Data Collection from Three Creeks in the Upper Subwatersheds of the Strawberry River, AR, Fulton Co, USA** T.R. Brueggem, Arkansas State Univ; J.L. Bouldin, Arkansas State Univ, Dept of Biological Sciences, Arkansas State Univ, Environmental Sciences Graduate Program, Environmental Sciences Graduate Program, Arkansas State Univ, Dept of Environmental Science. Agricultural activities continue to negatively influence surrounding waterways despite continual studies on the use of Best Management Practices (BMPs) to limit such impacts. A variety of BMPs studied are attempts to control soil erosion therefore limiting the harmful effects linked to excess sediment in waterways. These practices have been shown to be effective at preserving natural water quality. This four year study is focused on six collection sites, three in the upper portion of BMP implementation and three below BMP implementation, located in the upper subwatersheds of the Strawberry River, AR. Multiple BMPs including the exclusion of cattle from waterways while providing alternative water sources and use of no-till method to plant pasture grasses are being implemented. In this presentation, a summary of preliminary data collected from six months of intensive bi-weekly samples and two and half years of monthly samples will be summarized with a comparison between collection sites. Results from chemical testing include total suspended solids and nutrients including ortho-phosphates, nitrates and nitrites. Results from biological testing include *Escherichia coli*, WET testing using *Ceriodaphnia dubia* and *Pimephales promelas* and sediment testing using *Chironomus dilutus*. Significant aqueous toxicity, two lethal and two sublethal effects, was detected in four collections at a varying location each time. Significant sediment toxicity was detected in three collections with all but one site indicating either lethal or sublethal toxicity, at varying times. Additionally, two spring and two fall benthic macro-invertebrate analysis are compared. Results indicate the necessity of an array of long-term analyses to accurately assess the effectiveness of BMPs as results vary both spatially and temporally.

**RP268 Total Mercury Concentrations in Mexican Specimens of Sierra and Red Snapper** P. Ramirez Romero, Universidad Autonoma Metropolitana, Hidrobiologia, U.A.M. Iztapalapa, Depto. De Hidrobiologia; G. Solorzano-Ochoa, M. Ramirez-Islas, A. de la Rosa Perez, T. Ortuno-Arzate, Instituto Nacional de Ecologia, CENICA; J.E. Padilla Torres, J.G. Trejo Ramirez, Universidad Autonoma Metropolitana, Hidrobiologia. Mercury is potentially toxic and persistent and can be accumulated and biomagnified. Aquatic ecosystems are particularly vulnerable because mercury can be deposited as  $Hg^{2+}$  bounded to particles in suspension that are accumulated as sediments in lakes, lagoons and estuaries, where mercury can be integrated in the trophic chains and end up in organisms consumed by human beings. The objective of the present work was to evaluate the concentration of total mercury in specimens of sierra and red snapper captured in six coastal Mexican towns. Fish samples were obtained in 2009 and 2010 in Ensenada, Mazatlán, and Puerto Madero, on the Pacific Coast and Tampico,

Coatzacoalcos, and Puerto Progreso on the Gulf of Mexico. Lyophilized dorsal muscle was analyzed with an AA spectrophotometer; results were compared with international standards for human consumption. Red snapper (*Lutjanus campechanus*, *Lutjanus guttatus*) average Hg concentrations varied from 0.1 to 0.6 ppm with a few specimens above the Mexican quality criteria (1.0 ppm). Sierra (*Scomberomorus maculatus*, *Scomberomorus sierra*) mercury concentrations were low in general, with a maximum of 0.6 ppm. The comparison of red snapper average concentrations per year showed a higher mercury concentration in 2010 in Coatzacoalcos Mazatlan and Puerto Madero and for sierra only in the last two sites. Mercury concentrations in sierra and red snapper do not represent a risk for human health in accordance with the Mexican quality criteria, however the apparent trend towards higher concentrations from one year to the next makes it necessary to continue monitoring mercury in edible fish.

**RP269 Toxicity of Chemical Mixtures in Stormwater: Malathion and Benzene Toxicity to *Daphnia magna*** M. Cains; W.G. Landis, Institute of Environmental Toxicology, Western Washington Univ, Western Washington Univ, Institute of Environmental Toxicology, Western Washington Univ, Institute of Environmental Tox. & Chem. Stormwater is comprised of chemical mixtures from multiple nonpoint runoff sources. The increasing urbanization of land use causes contaminants on the impervious surfaces to be carried away in stormwater and into the nearest waterway. The common landscaping and agricultural practice of over-application of pesticides leaves the excess toxicant to be washed away with the field runoff water. While there is information on individual chemical toxicity, little is known about the toxicity of chemical mixtures. In order to manage risk, it is critical to understand how contaminated stormwater can affect the reproduction and survival of non-target organisms. In this experiment, 21-day chronic *Daphnia magna* toxicity tests were conducted using >98% reagent grade benzene and analytical grade malathion. The chemicals were tested at the following concentration ranges: malathion 0.035-3.00 µg/L and benzene 450 – 28000 µg/L. A mixture of benzene and malathion was also tested to evaluate the potential additive, synergistic, or antagonistic toxicity: malathion 0.035 – 3.0 µg/L in the presence of 7000 µg/L of benzene. No synergistic effect was observed from examination of the concentration-response curves. Concentration-response curves were used in the data analysis since they provide confidence intervals and reflect the current model on toxicant-receptor interaction. The survival and reproduction rate data collected can be used to construct an age-structured population model to determine how the chemicals would change the dynamics of a *Daphnia magna* population.

**RP270 Toxicity of Imidacloprid to Juvenile and Adult Ghost Shrimp: Implications for Control in Willapa Bay and Grays Harbor, Washington** C. Grue, Washington Cooperative Fish and Wildlife Research Unit, Univ of Washington, School of Aquatic and Fishery Sciences, Univ of Washington, School of Aquatic & Fishery Sciences; J. Grassley, J. Frew, A. Troiano, Univ of Washington. Carbaryl has been used to control burrowing shrimp within oyster beds in Willapa Bay and Gray's Harbor, Washington for six decades. These shrimp destabilize sediments onto which young Pacific oysters are seeded. Imidacloprid (IMI) may be a viable alternative to carbaryl for controlling burrowing shrimp. Its toxicity to non-target fishes is significantly less than carbaryl. However, efficacy has varied among substrates leading to the testing of application rates up to 2.0 lbs ai/ac. Data on the toxicity of IMI to the shrimp species of concern are lacking, preventing comparisons between toxic levels and concentrations of IMI found within sediments post application, and between the sensitivity of the target species and non-target invertebrates. In the first of two tests, we exposed juvenile ghost shrimp (*Neotrypaea californiensis*) to geometrically arranged concentrations of IMI (Nuprid® 2F: 23-1,500 ppb ai) in seawater (17±2 C, 30 ppt) encompassing the range of concentrations observed in sediments following applications to mudflats in the Bay. We used two replicates of 5 shrimp per concentration for 96 h under static conditions with a water renewal at 48 h. Impairment (immobilization) of shrimp increased with concentration and time, but mortality at the highest concentration (20%) was similar to that in controls (10%). In the second test, adult shrimp were exposed to the same concentrations (4 shrimp/replicate, 3 replicates/concentration) for 24 h; a more likely scenario given loss rates of IMI in sediment post application. Impairment of shrimp also increased with concentration and time, but none of the shrimp died. Results suggest ghost shrimp are not as sensitive to IMI as other invertebrates, and levels necessary to kill the shrimp directly may exceed concentrations in sediments associated with maximum application rates. The

potential for the observed overt “sublethal” effects to result in mortality due to other stressors needs to be investigated.

**RP271 Toxicity of Pesticides to African Clawed Frog (*Xenopus laevis*) Embryos and Larvae** S. Yu, M. Wages, Texas Tech Univ/TIEHH, Dept of Environmental Toxicology; G.P. Cobb, Texas Tech Univ, TIEHH, Environmental Toxicology. Amphibian declines have been reported world-wide and evidence suggests that environmental contaminants may be a contributing factor to this global phenomenon. Previous research has indicated that pesticide use related to agriculture is associated with population decline of some North American amphibians. Therefore, toxicity data are needed to assess the risk of pesticides to amphibian species, especially during the aquatic embryonic and larval stages. The African clawed frog (*Xenopus laevis*) is a standard model organism for toxicological study, and standard procedures have been developed to evaluate developmental toxicity of chemicals (FETAX test). However, *X. laevis* has been reported to be more tolerant to some contaminants compared with North American amphibians. Therefore, understanding the differential sensitivity is crucial to conduct risk assessment and conservation strategies for amphibians. The current study aimed to determine pesticide toxicity on *X. laevis* and to determine if *X. laevis* represents an appropriate model to evaluate the adverse effects of pesticides on North American species. We conducted 96 h acute toxicity tests with three commonly used pesticides, malathion, endosulfan, and  $\alpha$ -cypermethrin on *X. laevis* embryos and larvae. The acute median lethal concentrations (LC50s) were determined as well as malformation and growth. Malathion and endosulfan acute LC50s were estimated to be 4.5 mg/L and 1.4 mg/L, respectively, for embryos. For larvae, LC50s of all three pesticides were 5.4  $\mu$ g/L for  $\alpha$ -cypermethrin, 0.3 mg/L for endosulfan, and 5.9 mg/L for malathion. Malathion caused tail flexure, abnormal gut coiling, and edema in embryos and larvae, while endosulfan primarily produced tail flexure and edema. Similarly, tail flexure and edema were common in embryos and larvae exposed to  $\alpha$ -cypermethrin. Pesticide toxicity to *X. laevis* determined in the current study was compared with those reported for other amphibian species. In general, *X. laevis* is less sensitive to malathion and endosulfan than North American amphibians. It is unclear if *X. laevis* is also more tolerant to  $\alpha$ -cypermethrin because toxicological data for  $\alpha$ -cypermethrin are scarce.

**RP272 Toxicity of Two Herbicides to Two Life Stages of Bull Trout and Rainbow Trout** H.J. Puglis, USGS, Columbia Environmental Research Center; R.D. Calfee, USGS, Columbia Environmental Research Center, US Geological Survey, Columbia Environmental Research Center; E.E. Little, E.L. Beahan, USGS, Columbia Environmental Research Center. Eurasian water milfoil (*Myriophyllum spicatum*) is a highly invasive aquatic plant that has spread throughout aquatic habitats in the United States including western habitats of bull trout (*Salvelinus confluentus*). Bull trout populations are declining and the bull trout was listed as a threatened species by the US Fish and Wildlife Service in 1998. Aquatic herbicides are being considered as a method to eradicate water milfoil; therefore, it is particularly important to understand the sensitivity of bull trout to herbicides that might potentially be applied to control milfoil in their habitats. The toxicity of the herbicides Weedar® 64 (2,4-D) and Renovate® 3 (triclopyr) to bull trout were determined in 96-hour static acute exposures conducted at two stages of juvenile development. Preliminary studies have shown that 2,4-D and triclopyr LC50s are well above the maximum recommended application rates of both herbicides, indicating that bull trout are fairly tolerant. Similar studies were conducted with rainbow trout (*Oncorhynchus mykiss*), a potential surrogate species. Data from these studies will assist local, state and federal agencies protect the threatened bull trout, while eradicating water milfoil.

**RP273 Transfer of Mercury Across Ecosystem Boundaries in Arid Streams** A.A. Abuzeineh, J.R. Troy, Texas State Univ – San Marcos, Dept of Biology; M.M. Chumchal, Texas Christian Univ, Biology Dept; M.C. Green, Texas State Univ – San Marcos, Dept of Biology; W.H. Nowlin, Texas State Univ – San Marcos, Assistant Professor. Mercury (Hg) is a toxic element with no known biological function found in environments around the world. Through our activities, humans have greatly altered the global Hg cycle and subsequently released large amounts of Hg to the environment. Currently, it is estimated that 2/3 of the Hg cycling in the biosphere is anthropogenically derived. The bioaccumulation and transfer of Hg within food webs has largely been examined in aquatic ecosystems; however, ecotoxicologists and environmental scientists have recently focused

on the movement of contaminants across ecosystem boundaries. The input of Hg into aquatic systems occurs largely through atmospheric deposition of inorganic Hg, but the movement of aquatically-derived Hg to terrestrial environments is likely biologically mediated via the movement of organisms crossing ecosystems boundaries (e.g., movement of organisms from aquatic to terrestrial environments). In this study, we assessed the importance of Hg transport from lotic ecosystems via aquatic insect emergences to terrestrial consumers. We examined the Hg concentration in in-stream food web components (fishes and aquatic invertebrates) and in terrestrial consumers which forage in riparian zones, such as bats, birds and spiders. The study sites included two “High Hg” stream ecosystems which have known geological Hg and a past history of Hg mining in the watersheds, and one “Low Hg” stream with little geological Hg and no Hg mining in its watershed. Fishes and aquatic invertebrates at the “High Hg” sites generally exhibited higher Hg concentrations than at the “Low Hg” site. We found significantly higher Hg concentrations in feathers of both resident and migrant birds at the “High Hg” sites. However, we did not detect significant differences in Hg concentrations in insectivorous bats and terrestrial invertebrates among the “High” and “Low” sites. This study supports a growing body of evidence demonstrating the role that aquatic organisms play in movement of toxicants to terrestrial ecosystems.

**RP274 Trenbolone Acetate Metabolites May Promote Ovarian Growth in Adult Medaka** K.L. Forsgren, Univ of California, Riverside, Dept of Environmental Sciences; S. Qu, D.M. Cwierny, Univ of California, Riverside, Dept of Chemical and Environmental Engineering; D. Schlenk, Univ of California, Riverside, Dept of Environmental Sciences. Trenbolone acetate, a synthetic androgen, has been used as a growth promoter in beef cattle in the US since 1987. Three biological metabolites are detected in the manure of implanted cattle: 17a-trenbolone, 17b-trenbolone and trendione. 17a-trenbolone is excreted in higher amounts however is a less potent androgen receptor agonist than 17b-trenbolone or trendione. These metabolites have been detected in surface water associated with cattle feedlots. While several studies have investigated the masculinization effects of 17b-trenbolone, few have focused on the reproductive impacts in mature female teleosts. In the current study, adult female medaka (*Oryzias latipes*) were exposed to low (10 ng/L) and high (1000 ng/L) concentrations of 17a-trenbolone, 17b-trenbolone and trendione for 14 days (N = 3). Histological examination revealed that all treatments resulted in a lower percentage of primary ovarian follicles and a higher percentage of maturing follicles compared to control fish. Additionally, after 7 days of exposure, females exposed to 17b-trenbolone and trendione began laying eggs and continued to lay eggs daily throughout the remainder of the experiment. Whole body 11-ketotestosterone (pg/mg) did not differ significantly among treatments, whereas 17a-trenbolone (low and high) and the high dose of 17b-trenbolone significantly increased 17b-estradiol (pg/mg). In contrast, high dose treatment of trendione significantly decreased whole body 17b-estradiol (pg/mg). In vitro vitellogenin mRNA analysis using rainbow trout hepatocytes incubated with each metabolite was used to determine the estrogenicity of the three compounds. 17a-trenbolone (1000 ng/L) was equivalent to 0.25 ng/L estradiol (EEQ) whereas trendione (1000 ng/L) was 3 ng/L EEQ. Future studies will focus on elucidating the underlying mechanism(s) and pathway(s) of the reproductive and endocrine impairment caused by 17a-trenbolone, 17b-trenbolone and trendione.

**RP275 Triclosan Exposure to Bacteria Leads to Chlorine Tolerant Strains in Environmental Water Samples** J. Roling, S. Blackmon, Bridgewater State Univ, Biological Sciences. Triclosan is an antimicrobial agent added to a variety of medical and consumer care products such as soaps, deodorants, and cleaning supplies. These chemicals are then discharged into rivers, exposing native bacteria to triclosan. Bacterial exposure to triclosan could lead to chlorine resistance. Since chlorination is the standard method used to disinfect drinking water, municipal water sources may not be sterilized from native bacteria. Water samples were obtained downstream a wastewater treatment plant (WWTP) in Bridgewater, MA and reference virgin stream (VS) in Monroe, MA. Bacteria strains were isolated from water samples, exposed to triclosan (0.001 or 0.05mg/mL), and then exposed to chlorine (0.05mg/mL). 33% (112/336) of bacterial strains increased chlorine resistance after one triclosan exposure. 52% (87/168) of the WWTP bacteria strains increased chlorine resistance while only 15% (26/168) of the VS bacteria strains increased chlorine resistance. Although triclosan did increase chlorine tolerance, the triclosan concentration didn't influence the percentage of strains that gained chlorine resistance (38% and 35%



increase). Currently, agar plate assays are being conducted to verify these results.

**RP277 Use of Digital Photography as an Objective Aid in Mysid Shrimp Chronic Toxicity Tests** M. Foster; S. Garcia, Univ of North Texas, Biology; W.B. Steele, Univ of North Texas, Environmental Science; D.B. Huggett, Univ of North Texas, Dept of Biological Sciences. *Americamysis bahia* (mysid shrimp) are common saltwater toxicity test organisms. Currently, acute and chronic standard testing protocols for mysids are available, with a multi-generational protocol in development. Standard testing protocols require the counting and sexing of the organisms at multiple points throughout the duration of the experiments. Traditionally, the counting and sexing is performed with the naked eye by trained personnel, while the organisms are swimming inside water-filled containment chambers. With the aid of digital photography, accurate mysid counts can be made in a reduced time period when compared to the standard procedure. In our laboratory, organisms are counted and sexed using high resolution pictures taken from a nine megapixel standard digital camera mounted on a metal stand. The primary advantage of using digital photography for biological observations during toxicity tests is that the digital images reduce the lengthy one-on-one training required to quickly and accurately sex and count the organisms. Because digital photographs can be projected onto a larger TV screen or monitor, multiple trainees can be taught simultaneously, thus reducing the amount of time and money spent on training staff. Additionally, digital photography provides an empirical record of protocol identification number(s) that correspond to every test chamber in studies. These photos could be referred to for quality control assurance as well as provide permanent, objective evidence for GLP studies. The use of digital photography has important implications in aquatic invertebrate toxicity testing as this method may enhance the speed and accuracy of biological observations, reduce personnel training time, and improve quality control, all while providing a digital record of every experimental organism used in mysid studies.

**RP278 Use of Gene Expression in Longear Sunfish (*Lepomis megalotis*) and Green Sunfish (*Lepomis cyanellus*) as a Biomarker of Pollutant Exposure** B.F. Brammell, Asbury Univ, Natural Sciences Dept; D.K. Peyton, K.R. Barnett, J.C. Ferrell, Morehead State Univ, Dept of Biology and Chemistry; A.J. Wigginton, Univ of Kentucky, Dept of Civil Engineering. Biomarkers are effective monitoring tools, allowing researchers to assess physiological responses to pollution thereby contributing to both pollutant detection and an understanding of the biological significance of contamination. We obtained partial sequences of three biomarker genes in both longear sunfish (*Lepomis megalotis*) and green sunfish (*Lepomis cyanellus*), two widely distributed sunfish species. Partial sequences of metallothionein and uridine 5'-diphospho-glucuronosyltransferase for both species and cytochrome P4501A1 for green sunfish have been submitted to Gen Bank. Expression of these genes will be examined using real-time PCR in sunfish collected from contaminated and reference sites of streams surrounding the Paducah Gaseous Diffusion Plant (PGDP) in western Kentucky. The streams surrounding the PGDP that are the focus of this study have a long and well documented history of contamination by both organic and inorganic contaminants. Sediment PCB concentrations in contaminated sites at the time of fish collection were between 10.09 and 14.29 µg/Kg while PCB concentrations as high as 0.473 µg/g total PCBs were observed in fish tissue. Aqueous copper concentrations in contaminated sites at the time of fish collection were as high as 9.18 µg Cu/L, a level consistent with the induction of the metal sensitive protein metallothionein in similar studies. Gene expression analysis is in progress and will provide valuable information linking contaminant levels to biomarker response.

**RP279 Use of the Comet Assay for the Monitoring of Biological Effects of Petroleum Products in the Gulf of Mexico** M. Guzman Martinez, Universidad Autonoma Metropolitana, Dept of Hidrobiologia; P. Ramirez Romero, Universidad Autonoma Metropolitana, Hidrobiologia, U.A.M. Iztapalapa, Depto. De Hidrobiologia; L. Elizalde Ramirez, L. Espejel Pina, J.E. Padilla Torres, J.G. Espejel Trejo, Universidad Autonoma Metropolitana, Hidrobiologia; A.D. Nava Montes, Instituto Nacional de Ecologia, CENICA. Monitoring is an important tool in environmental management; it allows the evaluation of temporal and spatial tendencies of the environmental quality. Biomarkers can be used as tools for the evaluation of exposure and effects in aquatic ecosystems after an oil spill. The single cell electrophoresis method or comet assay evaluates damage to DNA. The

objective of this study was to establish a base line of DNA damage in oysters collected in the Gulf of Mexico, an area with high risk of being exposed to oil products. The oyster *Crassostrea virginica* was selected as sentinel organism. Specimens were sampled in Laguna de Terminos, which is located in the Campeche sound where the largest oil field in Mexico is located, and in Alvarado Lagoon where petroleum pollution has been reported in the past. Thirty organisms were collected at each site and brought to the laboratory in an ice box to keep them alive. DNA damage was evaluated using the comet assay technique; images were analyzed using the software Cromagen. The average length of the tails was determined and in this first sampling none or very low damage was determined (1-20 µm), which indicated that the area where the oysters banks are located have a good environmental quality at least during the dry season, when the sampling took place. The next step in this research will be to evaluate this biomarker during the rainy season when pollutants are carried to the sea through this lagoon system.

**RP280 UV Filters Endocrine Disruption and Neurotoxicity on the Tropical Damselfish *Abudefduf saxatilis*** M. Soto, Roger Williams Univ, Marine Biology, UNAM, Instituto de Ciencias del Mar y Limnologia; G. Rodriguez Fuentes, Centro para el Estudio del Agua, Unidad de Ciencias del Agua-CICY. Oxybenzone, Octylsalate and Octynoxate are compounds used in a variety of products as a protection against the UV radiation exposure. Concerns have emerged regarding their environmental safety. Previous studies have shown that they tend to bioaccumulate and act as potential xenoestrogens in aquatic organisms. Until now, nobody has investigated their impact on tropical reef associated species. Reef environments like the Mesoamerican Barrier Reef System are particularly sensitive to the introduction of novel contaminants. This study investigates the estrogenic effect of these three compounds on the abundant reef associated damselfish sergeant major *Abudefduf saxatilis*. Estrogenicity was evaluated using gene expression and protein synthesis analyses to monitor the induction of vitellogenin (VTG) in juvenile fish (< 5cm). Fish were exposed to doses of 5, 25 and 50µg/g of oxybenzone, octylsalate, octynoxate and to a mixture in a similar proportion to the one used in personal care products. Since neurotoxicity was observed during a preliminary bioassay, head cholinesterase activity (ChE) was also analyzed. As no genomic information on *A. saxatilis* was available, a first step of the work has been to isolate and sequence VTG, 18S and βactin genes. Although no clear expression of VTG gene was observed by qRT-PCR, all treatments induced the synthesis of the protein measured as Alkali Labile Phosphate (ALP) at levels similar to the positive control 17β-estradiol (E<sub>2</sub>) and xenoestrogens reported in other studies ranging between 5 and 10µg/ml. Interestingly, all treatments showed significant cholinesterase inhibition, the mixture showing the major neurotoxic impact with 48,8 ± 3,5% inhibition compared to the negative control. E<sub>2</sub> at a dose of 5µg/g, which is often used as positive control for estrogenic effect studies, also produced a cholinesterase inhibition of 54,5 ± 3,6 %. Changes in ChE activity by UV filters and E<sub>2</sub> may be linked to the production of reactive oxygen species (ROS), leading to oxidative stress. Further studies are conducted to evaluate the transcriptional pathway for VTG synthesis in *A. saxatilis*. This research shows that oxybenzone, octylsalate, octynoxate and their mixture have estrogenic effects on juvenile sergeant major. In addition, we also have demonstrated neurotoxicity. Focus on the estrogenic effect of these compounds may have led to underestimate other ecotoxicological impacts.

**RP281 UV Tolerance of High Elevation Copepods of the Colorado Rocky Mountains** K. Hudelson, Univ of North Texas, graduate student; B. Barst, Univ of Quebec INRS, Eau Terre Environnement; J. Smith, A. Roberts, Univ of North Texas. Copepods in high elevation lakes and ponds in Colorado are exposed to significant levels of ultraviolet radiation (UV), necessitating development of UV avoidance behavior and photoprotective physiological adaptations. The copepods are brightly pigmented due to accumulation of astaxanthin, a carotenoid which has photoprotective and antioxidant properties. Astaxanthin interacts with a crustacyanin-like protein, shifting the absorbance from 473 nm (hydrophobic free form, appears red) to 632 nm (protein-bound complex, appears blue). In six sites in Colorado, habitat specific coloration patterns related to carotenoprotein complex have been observed. The objective of this study was to determine whether pigment accumulation or carotenoprotein expression has a greater effect on resistance to UV exposure. For each site, copepod tolerance to UV was assessed by survivorship during UV exposure trials. Control organisms were exposed only to the visible spectrum (shielded from UV). Average UV exposure was determined for each habitat. Astaxanthin profiles were generated



for copepods in each site. Ability to withstand UV exposure during exposure trials was significantly different between color morphs ( $p < 0.0001$ ). Red copepods were found to tolerate 2-fold greater levels of UVB than blue or mixed copepods. Additionally, red copepods have much higher levels of total astaxanthin than blue or mixed copepods ( $p < 0.0001$ ), and receive a higher daily UV dose ( $p < 0.0003$ ). Carotenoprotein expression may be an important adaptation to changing UV conditions and may serve as an indicator of increasing UV exposure in high elevation lentic ecosystems.

**RP282 Global Guidance Principles for LCA Databases** B. Vigon, SETAC; G. Sonnemann, United Nations Environment Programme. In early 2011 participants from 23 countries gathered in Japan for a Pellston workshop to develop global guidance on principles to create, manage, and disseminate datasets for the purpose of supporting life cycle assessments of products and services. The vision for the workshop was to create guidance that would: 1) serve as the basis for improved dataset exchangeability and interlinkages of databases worldwide, 2) increase the credibility of existing LCA data, the generation of more data, and enhance overall data accessibility, and 3) complement other data-related initiatives at the national or regional level, particularly those in developing countries and where more prescriptive guidance has been developed. In addition to providing guidance on technical and operational aspects of datasets and databases, it was discovered that there remain differences in terminology usage and inconsistencies in principles definitions. Part of this situation is caused by the evolution of LCA in different regions/cultures, part by language and part by ambiguity in existing definitions. Thus, one exercise was to develop a terminology glossary and dictionary of principles to provide a basis of reference for participants. Much time and effort was spent assessing the current state-of-practice regarding developing datasets, incorporating them into databases, and then managing those databases. From an operational standpoint, recognition that the target audience of the document is database managers serves to position them as central actors in the data supply chain. Data documentation and review are key elements. Database managers have the role and responsibility to decide not only what the datasets themselves must include but also what additional information is required and what would be recommended or necessary in terms of validation and review. Some workshop participants identified a need for additional data and data management to allow LCA databases to provide more comprehensive answers and to answer more comprehensive questions, such as spatially differentiated models, time-based methods, and issues related to social and economic impacts. Another aspect addressed was the filling of data gaps with data estimations from non-process based approaches. Lastly, one group developed future scenarios on how data and databases could work in concert with the growth of LCA globally and anticipating advances in information technology in the next several years.

**RP283 Environmental Assessment of an Integrated Greenhouse Tomato Crop Grown Under Northern Conditions** M. Dorais, Agriculture and Agri-Food Canada, Sustainable Production Systems; A. Anton, J. Montero, M. Torrellas, IRTA Carretera de Cabrils. The environmental burdens associated with fossil energy use, in addition to water and fertilization management continue to be major concerns for Northern greenhouse production systems. To reduce the environmental impacts of greenhouse farming under Northern climate conditions and to maintain its competitiveness, the use of renewable energy sources and suitable nutrient and waste management are becoming essential. From this perspective, a closed-loop organic production system that increases the efficiency of water and nutrient use on-farms and utilization of waste biomass was developed and tested in the province of Quebec, Canada. The goal of this study was to assess the environmental impacts of this integrated system compared to a conventional system in order to use its environmental profile as a starting point for improving the sustainability of existing systems. The environmental analysis was conducted with LCA methodology as defined by the ILCD handbook (2010) and the SimaPro v.7.3 software. The functional unit was 1000 kg of tomatoes. The system boundary was from raw materials extraction to the farm gate. The life cycle stages considered were the infrastructure, auxiliary equipment, climate control system, farm operation, fertilizers, pesticides, waste management and packaging. Results from the environmental assessment indicate that high energy demand had the major contributions to all impact categories when fossil energy is used. When biomass was used as renewable energy, biomass transport became the most important environmental impact factor. Increasing the truck capacity as well as reducing the distance from the farm to the biomass source reduced environmental burdens related

to energy. Greenhouse structure made the second highest contributions to environmental impact categories. For the organic crop scenario, fertilizers and biopesticides had a negligible impact. Results are discussed in terms of potential alternatives towards zero emission and to reach energy neutral for a Northern greenhouse production system.

**RP284 ISO- and Ecologically-based Life Cycle Assessments of Palm Oil Biodiesel** Y. Manik, Univ of Maine, Research Group of Industrial Ecology, LCA and System Sustainability (IELCASS); A. Halog, Univ of Maine, Research Group of Industrial Ecology, LCA and System Sustainability (IELCASS), Univ of Maine, Assistant Professor of Industrial Ecology and LCA. In order to assess the sustainability of biodiesel derived from palm oil (*Elaeis guineensis*), two different but complementary life cycle assessments (LCA) have been performed: The attributional LCA based on ISO-14040 standard and the Ecologically-based LCA (Eco-LCA) based on the methodology recently developed in Ohio State Univ. The attributional LCA is a *cradle-to-grave* study seeking to investigate the environmental impacts of palm oil biodiesel life cycle from land preparation for oil palm plantation to biodiesel utilization in passenger cars. The results show that palm oil biodiesel has significant life cycle impacts in global warming and eco-toxicity potentials. The conversion of forest land and methane emission from anaerobic digestion of palm oil mill effluent and energy use in various processes are the most significant processes contributing to global warming potential. The use of herbicides and pesticides in plantation that emits glyphosate and cypermerterin to the air and agricultural soil significantly contribute to ecotoxicity potential. An Eco-LCA study was performed in order to extend the comprehensiveness of the sustainability assessment taking into account goods and services from ecosystem. The aggregation of ecological inputs were classified based on mass, energy and industrial cumulative exergy (I exergy). The results show the reliance of palm oil biodiesel to the natural capital provided by the ecosystem, including solar energy, carbon dioxide from atmosphere, detrital matter from biosphere, water from hydrosphere, as well as fossil fuels and various form of mineral from lithosphere which are not shown yet in the attributional ISO-LCA.

**RP285 Modeling the Development of Greenhouse Gas Effects on Eco-industrial System Through Life Cycle Approach for the Pulp and Paper Industry in Maine** N. Bichraoui, Univ of Maine, School of Forest Resources, Univ of Maine, School of Forest Resources; A. Halog, Univ of Maine. This research aims to pursue the vision of future closed loop industrial ecosystems and to develop dynamic scenarios on how to achieve this vision technologically. The method will include a holistic approach to assess the development of low carbon emissions and low water consumption within an industrial network. The research will focus on pulp and paper industry in the state of Maine. Just like other industries, the pulp and paper industry has come under increasing scrutiny for its potential environmental impacts. More than many other industries, however, this industry plays an important role in sustainable development because its chief raw material – wood fiber – is renewable. The industry provides an example on how a resource can be managed to provide a sustainable supply to meet society's current and future product needs. In 2001 Maine was the second largest manufacturer in the US as measured by volume, with roughly 4.5 billion tons in production. In the Northeast Maine is clearly the dominant paper producer.[1] This study is intended as reveal alternative processes to improve production and consumption process with lower environmental impacts. The life cycle assessment method is therefore appropriate because it allows the performance of a holistic analysis of pulp production that takes into account the extraction and processing of raw materials, the manufacturing processes, transport, use, reuse and, finally, recycling and disposal at the end of life. The intended audience consists of this study are forest product professionals along with LCA practitioners interested industry and in getting valuable insights in environmental burden for this specific industry. The modeling tools of system dynamics, materials flow analysis and agent-based modeling will be further used in order to capture the complexity of the system and explore the future scenarios of this industrial ecosystem with respect to the human dimension and its impact on normal events such as new actors emerging, takeovers and the introduction of innovative (and sustainable) technologies and policies [1] Maine forest Future Economy Project, Final report (Mars 2005), Innovative Natural Solutions

**RP286 Comparison of the Effects of Carbon Nanotube and Asbestos Exposure on the Immune System of Fathead Minnows** K.B. Donohue,

J.G. Laird, D.R. Johnson, J.K. Stanley, J.A. Steevens, US Army Engineer Research and Development Center, Environmental Laboratory; A. Edgington, S.J. Klaine, Clemson Univ, College of Agriculture, Forestry and Life Sciences. Carbon nanotubes (CNT) are widely used in various military, commercial and public sectors; e.g., electronics, aerospace, computer industries, consumer products (sunscreens and cosmetics) and medicine. The unique nano structure of CNT is based on a graphene cylinder, typically a few nanometers in diameter, which can range in length from micrometers to millimeters. Single-walled carbon nanotubes (SWNT) consist of one such cylinder while multi-walled carbon nanotubes (MWNT) consist of 2-50 such concentrically stacked cylinders with a long common axis resulting in fiber-like characteristics in terms of their elongated shape, dimensions and high aspect ratio (ratio of length and width). The needle-like fiber shape of CNT has been compared to that of asbestos, raising concerns about potential toxicity. The objective was to compare the immunological effects of CNT exposure to that of asbestos exposure in *Pimephales promelas*, commonly known as fathead minnows (FHM). FHM were exposed to 0.125mg/L, 0.25mg/L, or 0.5mg/L CNT (MWNT and SWNT) or asbestos (crocidolite and chrysotile for MWNT and SWNT, respectively) in water that was renewed daily. At days 0, 7, and 14, FHM were sacrificed and mucus swabs, blood, and anterior kidneys were collected. Plasma serum was separated from blood cells and used in ELISA assays. Lymphocytes were purified from both blood cells and anterior kidneys by density gradient centrifugation. Lymphocytes were then probed for phagocytic activity using a phagocytosis assay kit. Inflammasome activation was measured by ELISA for IL-1 $\beta$ , one of the first cytokines processed by the inflammasome. Antibody production was measured by ELISA for IgM. Exposure to CNT resulted in decreases in phagocytic activity, mucus antibody production and plasma serum antibody production compared to untreated controls. Asbestos exposure also decreased mucus antibody production but not plasma serum antibody production. Curiously, exposure to chrysotile asbestos resulted in a decrease in induced phagocytosis but not in un-induced phagocytosis at day 14. Inflammasome activation, as measured by IL-1 $\beta$  ELISA, was mostly undetectable; likely indicating that IL-1 $\beta$  processing occurs prior to day 7. Together, these data suggest that like asbestos exposure, exposure to CNT results in an immune response. However, the data demonstrate that further characterization of the systemic immune response to CNT is needed to assess the severity and toxicological impact of CNT.

**RP287 Determination of Nanosilver Dissolution Kinetics in an Aqueous Medium** A. Harmon, US Army Engineer Research and Development Center, Environmental Laboratory; A.J. Kennedy, A. Poda, US Army Engineer Research and Development Center; A.J. Bednar, US Army Engineer Research and Development Center; B.D. Meeks, Badger Technical Services. Over the past few years there has been an increase in the manufacturing of nanomaterials. This increase in production of nanomaterials increases the potential for nanomaterials to be released into the environment. There are many unanswered questions regarding the fate of nanoparticles once they enter into the environment and the effect they may have on biological systems. The objective of this research is to determine the rate at which nanosilver will dissolve in environmentally relevant waters. The results from this research will lay the groundwork for fundamental interaction studies that will be performed to parameterize nanoparticle behaviors. Dissolution is a critical process that determines nanosilver effects in the environment and within organisms. Diluted suspensions of nanoparticles with moderately-hard reconstituted water (MHRW) were created from stock solutions in milli-Q water and placed on a shaker table for 0, 0.25, 1, 2, 7, and 14 days. Dilutions of 10% and 50% MHRW were used as the medium. Upon removal from the shaker table total silver measurements and dissolved silver measurements (ultra centrifugation, followed by ICP-MS analysis) were taken. Dynamic Light Scattering (DLS) and Field Flow Fractionation (FFF) were used to determine particle size at each time points by measuring the relative change in particle size over time. Nanocompsix NC80 biopure nanosilver was used in this experiment. In concentrations of 10% and 50% MHRW nanoparticles increased in particle size as time increased. In 10% MHRW particle size ranged from 82.7-91.3 and 91.6-167.7 in 50% MHRW. In days 0-2 there is more total silver than dissolved silver in samples. In days seven and fourteen there is more dissolved silver than total silver possibly due to the crashing out of particles. This research suggests that initially the concentration of dissolved silver will increase once in environmentally relevant media, but the longer term equilibrium between the

nanoparticle and dissolved fractions and associated biological effects remains poorly understood and further research is needed.

**RP288 In Vitro and In Vivo Effects of Single-walled Carbon Nanotubes Functionalized with Single Strand DNA Over Redox Balance** T. Mocan, A. Muresan, A. Filip, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Physiology Dept; A. Biris, National Institute of Molecular and Isotopic Technologies, Cluj-Napoca, Romania, Hydrogen Dept; S. Simon, National Institute of Molecular and Isotopic Technologies, Cluj-Napoca, Romania, Hydrogen Dept; D. Daicovicu, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Physiology Dept; N. Decea, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Clinicilor 1; R. Moldovan, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Physiology Dept; F. Tabaran, Univ of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania, Morphopathology Dept; A. Stir, Institute of Gastroenterology and Haepatology, Cluj-Napoca, Romania, Clinicilor 1; L. Mocan, Institute of Gastroenterology and Haepatology, Cluj-Napoca, Romania, Nanomedicine Dept; C. Catoi, Univ of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania, Morphopathology Dept; S. Clichici, Univ of Medicine and Pharmacy, Cluj-Napoca, Romania, Physiology Dept. Single wall carbon nanotubes (SWCNT) represent a material of high medical interest. Recent in vitro studies showed increased oxygen free radical production after cells exposure to SWCNT. However, reports regarding in vivo effects of SWCNT over redox balance as well as concentration effect over in vitro and in *vitro* SWCNT toxicity are still scarce. Our aim was to evaluate the in vitro and in vivo induced by SWCNT functionalized with single strand DNA(ssDNA-SWCNT) solutions. Material and methods: ss-DNA-SWCNT water solution was obtained through sonication. In vitro experiments were carried out on Hep G2 cell line. 5, 10 and 20 mg/L concentrated solutions were prepared. MTT assay and 5-(and-6)-carboxy-2',7'-dichloro-fluorescein diacetate (DCFDA) assay were performed. In vivo experiments were carried out on male Wistar rats (170 $\pm$ 10g), i.p. injected with 1.5 ml single walled ss-DNA-SWCNT solutions of different concentrations (70g/l, 250 g/l, 390 g/l). Both acute exposure( 1 ip. injection of 1.5 ml solution ) and repetitive exposure models were designed. Controls were similarly i.p injected with 1.5ml serum. Malondialdehyde (MDA), carbonylated proteins (PC), hydrogen donor ability (HD), sulfhydryl groups (SH) were assessed in blood at 3, 6, 24 and respectively 48 hours after the SWCNT administration. Results: We obtained a significant in vitro and in vivo alterations of oxidative balance peaking at 24 hours from administration. ( $p < 0.05$ ). Results show significant dependence of observed effects on concentration. At 48 hours from exposure, levels of the analyzed markers remained altered. Repetitive exposure induces enhances oxidative stress. However, effect in not correlated with the number of repetitive administrations. Conclusion: Our results support the ability of ss-DNA-SWCNT to generate oxidative stress, the pattern of alterations depending on the concentration of SWCNT solutions. Present work was supported by Romanian National Research Council Grant NANOCITOX 42112/2008.

**RP289 Is the Size the Most Decisive Factor in Nanotoxicity of TiO<sub>2</sub> Nanoparticles in Fish?** O. Zapata-Perez, Cinvestav Unidad Merida, Secretaria Academica. Recently there has been a concern for small particles in the nano scale and their potential effects in the aquatic environment. Nanomaterials have been increasingly used in industrial production and daily life products. The study of the toxicological aspect of nanoparticles has taken strong interest among scientists around the world. One of the concerns in the nanotoxicological world is the characterization of the nanoparticles; in the present study we synthesize the nanoparticle with the size that we required (7 nm, 14 nm y 21 nm). There are huge gaps associated to the knowledge regarding the toxicity of these new nanoparticles in fish. The challenge for toxicological studies including this one is to identify key points and the possible effects of these nanoparticles, leading to propose experiments to better predict the toxicity and behavior of this and other particles in aquatic and tropical environments. In the present study, nano-TiO<sub>2</sub> was selected as the most representative nanomaterial and thus we evaluated the gene expression related to oxidative stress analyzing Catalase, Super Oxide Dismutase and Glutathione-S-Transferase, in juvenile male tilapias (*Oreochromis niloticus*). We exposed fish to 1.0 mg/L solution with different sizes of TiO<sub>2</sub> nanoparticles. Later on, fish were sacrificed at 3, 6, 12 and 24 h after treatment, and liver were excised. Total RNA from liver was extracted using the Trizol method and cDNA synthesis was performed using oligo-dT anchorage primers. Reactions were analyzed using Taqman probes for each



gene and gene expression was determined using a Real Time Polymerase Chain Reaction. Our results show significant differences among nanoparticle sizes, suggesting that size is a decisive factor regarding organism toxicity, since the smaller nanoparticles showed a higher gene expression.

**RP290 Oxidative Stress Signaling Pathways Involved in Toxicity of Silver Nanoparticles and Titanium Dioxide Nanoparticles in the Soil Nematode *Caenorhabditis elegans*** H. Eom, S. Park, J. Ahn, D. Lim, P.G. Nair, Y. Chung, Univ of Seoul; J. Yi, Seoul National Univ; J. Choi, Univ of Seoul, School of Environmental Engineering. Oxidative stress has often been reported as one of the most important toxicity mechanism related to nanoparticles (NP) exposure, however, most of the studies have been conducted in mammalian models. In the present study, toxicity of silver nanoparticles (AgNP) and titanium dioxide nanoparticles (TiO<sub>2</sub>NP) was investigated in the soil nematode, *Caenorhabditis elegans*, focusing on the involvement of signaling pathways related to oxidative stress. LC50s of AgNPs and TiO<sub>2</sub>NPs were first estimated and worms were subsequently exposed to sublethal concentrations of both NPs for global gene expression analysis using microarray. Microarray results suggest oxidative stress as an underlying mechanism of NPs in *C. elegans*. Reactive oxygen species (ROS) formation was then tested as a direct evidence of oxidative stress in NPs exposed *C. elegans*. The upstream signaling pathway activated in response to NPs exposure was investigated, paying special attention to the *C. elegans* PMK-1 P38 mitogen-activated protein kinase (MAPK) pathway. The expressions of the downstream genes and transcription factors, known to be regulated by the PMK-1 P38 MAPK was also investigated in *wildtype* (N2) and *pmk-1* loss-of-function mutant (*km25*) *C. elegans* exposed to AgNPs and TiO<sub>2</sub>NP. The overall results indicated that AgNPs and TiO<sub>2</sub>NPs exposure lead to increased ROS formation, PMK-1, GST activations and reproduction failure in *wildtype* (N2) *C. elegans*; whereas, none of these phenomena were observed in the *pmk-1* (*km25*) mutant. These results suggest that oxidative stress is an important mechanism of AgNPs and TiO<sub>2</sub>NP induced toxicity in *C. elegans*, and PMK-1 P38 MAPK plays an important role in it.

**RP291 Particle Size and Coatings of Nano Silver in Relation to Bio-availability and Particle Compartmentalization Within *Lumbriculus variegatus*** J.G. Coleman, US Army Engineer Research and Development Center, US Research and Development Center, Research Biologist; A.J. Kennedy, US Army Engineer Research and Development Center; J. Williams; J.D. Laird, US Army Engineer Research & Development Center, SpecPro Incorporated, US Army Engineer Research & Development Center, Environmental Laboratory; A.J. Bednar, US Army Engineer Research and Development Center; A. Harmon, US Army Engineer Research and Development Center, Environmental Laboratory; J.A. Steevens, US Army Engineer Research & Development Center, Waterways Experiment Station. As the production and applications of nano silver increase, it is essential to characterize fate, transport, and effects in environmental systems. Our objective was assess the fate of nano silver in aquatic environments and determine particle transport, location, and bioaccumulation in the freshwater oligochaete, *Lumbriculus variegatus*. To assess aquatic fate, we performed a series of dynamic light scattering (DLS) measurements on various particle suspensions. Suspensions were created in serial dilutions of freshwater media in a range of electrical conductivities (0-280  $\mu$ S/cm) to assess aggregation potential. Suspensions included 30, 1500 nm particles and a 20-30 nm polyvinylpyrrolidone (PVP) coated silver. Aggregation in 30 and 1500 nm silver increased with conductivity; however, aggregation was minimal for PVP coated silver. *L. variegatus* were exposed to nano-silver or silver nitrate (AgNO<sub>3</sub>) spiked into sediment (nominally 100 mg/L, n=3) to assess uptake and fate within biological compartments (i.e., particle location in gut wall or tissue). Time points included 2, 4, 7, 14, and 28 days in which *L. variegatus* were removed for body burden analysis and hyperspectral imaging. Additionally, *L. variegatus* were depurated for 6, 8, and 24 hours to determine time needed for gut-clearance time of nano-silver. Image analysis of *L. variegatus* cross-sections utilizing Cytoviva® hyperspectral microscopy detected PVP particles within sections. Bioaccumulation data from field flow fractionation and ICP-MS indicated an increasing trend in uptake; further, analyses indicated nano-silver in the organism was in particle form. While body burdens of nano-silver were higher in 1500 nm relative to 30 nm treatments early in the exposure, body burdens for 30 nm particles were higher relative to 1500 nm after 28-days exposures (4.57 and 3.35 mg/kg, respectively). The present study provides information on the potential for nano particle size/coatings to affect distribution within environmentally

relevant waters and provides information on particle transport across biological membranes.

**RP292 Silver Nanoparticle Toxicity is Associated with Oxidative Dissolution and Strongly Mitigated by Natural Organic Matter** X. Yang, Duke Univ, PHD student; J.N. Meyer, Duke Univ, Nicholas School of the Environment; H.H. Kim, Duke Univ, Civil & Environmental Engineering; A. Gondikas, Duke Univ, Pratt School of Engineering. The rapidly increasing application of silver nanoparticles (Ag NPs) in consumer products and medical applications has raised both ecological and human health concerns. A key question for addressing these concerns is whether Ag NP toxicity is mechanistically unique to nanoparticulate silver, or if it is a result of the release of silver ions. Using *Caenorhabditis elegans*, we have tested a series of differentially coated Ag NPs and found a linear correlation between Ag NP toxicity and oxidative dissolution, but no correlation between size and toxicity. Oxidative dissolution was a key to the toxicity of most Ag NPs, highlighting a critical role for dissolved silver in the toxicity of all tested Ag NPs. Pharmacological rescue and mutant analysis experiments supported these results. Some Ag NPs (typically less soluble due to size or coating) also acted via generation of reactive oxygen species, an effect specific to nanoparticulate silver. However, in no case was the toxicity of any Ag NP greater than would result from complete dissolution of the same mass of silver. We also studied the impact of natural organic matter (NOM) on Ag NP toxicity and found that NOM significantly mitigated Ag NP toxicity.

**RP293 Speciation of Atmospheric Particulate Matter in the Los Angeles Basin: Relating Specific Particulate Matter Components and Oxidative Stress Markers** D.A. Perkins, Univ of Wisconsin – Madison, Wisconsin State Lab of Hygiene; D.S. Antkiewicz, Univ of Wisconsin – Madison, Wisconsin State Laboratory of Hygiene, Wisconsin State Lab of Hygiene, Environmental Toxicology; J. Hemming, WI State Lab of Hygiene, Environmental Toxicology; M.M. Shafer, Univ of Wisconsin-Madison, Environmental Chemistry & Technology; J.J. Schauer, Univ of Wisconsin-Madison, Environmental Chemistry and Technology Program. Air pollution in the form of atmospheric particulate matter (PM) is a major concern in urban areas and has been linked to adverse human health effects such as asthma, bronchitis, respiratory infections, cardiovascular disease and cancer. An inflammatory response in pulmonary tissues can occur upon exposure to PM due to the formation of excess Reactive Oxygen Species (ROS). Alveolar macrophages are the primary pulmonary cells that respond to PM exposures by phagocytizing particles as well as producing ROS, contributing to the inflammatory reaction. PM species implicated in ROS formation include transition metals, organic carbon and specific organic species, e.g., PAHs and quinones. However, many advances are still needed to fully characterize the role and specific activity of PM components. To identify ROS active species and unravel the mechanisms of ROS/oxidative stress generation, we applied three different assays to water extracts of urban PM samples collected in the LA Basin, CA: an in vitro rat alveolar macrophage respiratory burst assay using the ROS fluorescent probe DCFH-DA, a dithiothreitol (DTT) assay measuring chemical redox activity by monitoring the oxidation of DTT, and an ELISA measuring rat tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) as a marker of inflammatory response. To assess the role of specific PM components, we coupled these assays with upfront chemical fractionation tools including Chelex (a metals chelator); an ion-exchanger for anionic metals; a dissolved organic matter sorbent; and an iron chelator (DFO). These methods were applied to both unfiltered and 0.2  $\mu$ m filtered PM extracts. The mass-normalized macrophage-ROS activity of urban PM varied substantially (up to 5 fold) between different LA basin urban environments. Soluble species contributed the majority of cellular ROS activity, though particulate activity was significant at several sites and represented a greater fraction of DTT activity. Chelex, DEAE and iron chelation treatments were effective in reducing ROS activity, however the magnitude and relative effect differed substantially from site to site. No significant decrease in respiratory burst was observed with soluble organic matter removal. In contrast, removal of organic species had an effect on TNF $\alpha$  production. The drivers of toxicity of atmospheric PM are complex and site-specific and reflect the unique PM composition rather than total PM mass.

**RP294 The Effects of Silver Nanoparticles on Antioxidant and Oxidative Damage Biomarkers Related to ROS Production in Isolated Tissues and Whole Oysters** B. Johnson, Univ of North Carolina-Charlotte, Dept of Biology, UNC Charlotte, Biology Dept; S. Gilbert, Univ of



North Carolina at Charlotte, Univ of North Carolina at Charlotte, Dept of Biology; M. McCarthy, UNC-Charlotte; L. Marsden, NC State Univ; N. Levi-Polyachenko, Wake Forest Univ Health Sciences, Dept of Plastic and Reconstructive Surgery; A. Ringwood, Univ of North Carolina Charlotte. The continued manufacturing and use of engineered nanoparticles makes it increasingly important to examine their effects on marine organisms. The purpose of these ongoing studies is to characterize the toxicity of various metal nanoparticle preparations on oysters, *Crassostrea virginica*. Studies are being conducted through specific isolated tissue exposure and also whole oyster exposures. Oyster hepatopancreas and gill tissue were isolated and exposed to the three types of silver nanoparticles (seeds, prisms, and plates) over a range of concentrations. Antioxidant (GSH), lipid peroxidation (LPO), and reactive oxygen species (ROS) levels were then measured. Whole oysters were also exposed to a range of silver nanoparticle concentrations to compare the responses in isolated tissues to those from whole oyster exposures. Silver nanoparticles caused increases in LPO and ROS production in oyster hepatopancreas tissues, and there was evidence of shape dependent differences in toxicity. In contrast, the gills were not affected as much as the hepatopancreas tissues in isolated as well as whole oyster studies. While isolated tissues are valuable assay systems for studying nanoparticle effects, there were some differences between the whole oyster studies and the isolated tissues studies. These kinds of basic studies are essential for addressing the potential impacts of nanoengineered particles on fundamental cellular processes as well as marine organisms.

**RP295 Uptake and Retention of Quantum Dots in *Daphnia* is Influenced by Particle Charge** A. Feswick, Univ of New Brunswick, Biology; R.J. Griffitt, Univ of Southern Mississippi, Gulf Coast Research Laboratory, Univ of Southern Mississippi, Coastal Sciences; K. Seibein, Univ of Florida, MAIC; D.S. Barber, Univ of Florida, Dept of Physiological Sciences. Nanomaterials are a diverse group of compounds whose inevitable release into the environment warrants study of the fundamental processes that govern ingestion, uptake and accumulation of nanomaterials in organisms. Studies have already demonstrated the ability of nanomaterials to transfer to higher trophic levels in aquatic ecosystems. Uncharged (PEG), positively charged (amino-terminated) and negatively charged (carboxyl-modified) cadmium selenide/zinc sulfide quantum dots were used to monitor ingestion, uptake and depuration of nanometals in *C. dubia* over 24 hours of exposure. These studies demonstrated that particles with higher negative charge (carboxy QDots) were taken up more readily by *Daphnia* ( $259.17 \pm 17.70$  RFU/20 *Daphnia* given as mean  $\pm$  S.E.) than either the amine ( $150.01 \pm 18.91$ ) or PEG Qdots ( $95.17 \pm 9.78$ ). This correlated well with fluorescent microscopic images obtained at the 24 hour timepoint. Confocal and electron microscopic analysis clearly demonstrated that all three types of quantum dots could cross the intestinal epithelial barrier and be translocated to other cells. Upon cessation of exposure, elimination of all three materials was biphasic with rapid initial clearance that likely represents elimination of material remaining in the GI tract followed by a much slower elimination phase that likely represents elimination of internalized material. These studies clearly demonstrate that daphnids can take up intact nanomaterials from the water column and that this uptake is strongly influenced by particle charge.

- Aars, Jon WP075  
 Abad, Esteban 717  
 Abalos, Manuela 717  
 Abazinge, Michael WP242  
 Abbott, Barbara TP053  
 Abbott Chalew, Talia 349  
 Abdallah, Mohamed MP127  
 Abdelrhman, Mohamed 161  
 Abe, Yasuhiro TP184, TP185, TP188  
 Abma, Rachel TP191  
 Abrell, Leif TP003  
 Abril, Sandra MP077  
 Abriola, Linda 735  
 Abusaba, Khalil MP133  
 Abuzeineh, Alisa RP273  
 Achayapunwanich, Orasa WP240  
 Achour, Farid 671  
 Ackerman, Janet 304  
 Acosta-Martinez, Veronica TP189  
 Adamec, Jiri 770  
 Adams, Douglas TP105, TP196  
 Adams, Jeff 235  
 Adams, Julie 448, WP077  
 Adams, William 232, 413, 673  
 Adedeji, Olufemi MP151, MP152  
 Adedigba, Bilqees MP097  
 Adekola, Adebayo TP082  
 Adekola, Folahan RP243  
 Adeyemi, Joseph RP255  
 Adiele, Reginald WP215  
 Adler, Nicole TP166  
 Adolfsson-Erici, Margaretha 205  
 Adrados, Miquel Angel 717  
 AduKumi, Sam 371  
 Aeppli, Christoph 212  
 Afrooz, ARM Nabiul RP082  
 Afzal, Atefeh WP043  
 Agbo, Stanley Ozoemena TP124  
 Agler, Matthew 710, 711  
 Agostinho, Angelo MP092  
 Aguilar Lazaro, Lisette 716, MP114  
 Agusa, Tetsuro 707  
 Aherne, Julian TP223  
 Ahlf, Wolfgang RP036  
 Ahmed, Kazi Matin 351  
 Ahn, JungMin RP290  
 Ahn, Sungwoo 757  
 Ahnell, Arden 210, 211, TP027  
 Ahrens, Lutz 65, 255, TP120  
 Aikawa, Bio 149  
 Aiken, George 38  
 Aitken, Michael TP163  
 Akase, Takanori TP188  
 Akerman, Gun 205  
 Akita, Tetsuya RP251  
 Akkanen, Jarkko 326, 660, WP067, MP100, TP124  
 Akuffo, Valerie WP279  
 Al-Reasi, Hassan 406  
 Alae, Mehran 195, 196, 197, 199, MP070  
 Alansari, Ahmed TP247  
 Alarcon-Herrera, Maria 350  
 Alava, Juan 498  
 Alban, Lepaillieur MP001  
 Albanese, Katie RP049  
 Albertus, Randal MP196  
 Albright, Vurtice MP232  
 Albuquerque, Anjaina 152, WP076  
 Aleksa, Katarina MP125  
 Alexander, Alexa 665  
 Alexander, Theresa MP161  
 Ali, Nadeem 176, MP126  
 Alix, Anne 9, 12, MP062, MP064, MP065  
 Alkema, Kyle MP140  
 Allam, Bassam 237  
 Allan, Sarah WP002, WP068, TP246  
 Allard, Cody TP058  
 Allard, Patrick 189  
 Allen, Herb TP100  
 Allen, Herbert 387, TP099  
 Allen, Joel WP164  
 Allen, Linda 187  
 Allen, Rachel TP216  
 Allert, Ann WP107, WP117, RP143  
 Alley, Bethany RP164  
 Allison, Paul 703  
 Alloy, Matthew WP168  
 Almaas, Kjersti TP021  
 Almeida, Marcos 712  
 Alpuche Gual, Leticia 258, WP246, TP255  
 Alsop, Derek WP210, RP219, WP225  
 Altin, Dag 313, 609, 767, RP094, WP140  
 Aluru, Neel 634  
 Alvarez, David 344, 345, 441  
 Alvarez, Natalia TP253  
 Alvarez-Chavez, Clara-Rosalia MP089, MP090  
 Alves, Debora WP076  
 Alvina Mehinto, Alvina 317  
 Aly, Alaa 294, TP173  
 Amagai, Takashi TP162  
 Amaral, Maria Jose 415  
 Amberg, Jon 18  
 Amcoff, Patric 329  
 Amos, Joshua MP183  
 Amstatter, Katja 760  
 Amundsen, Carl 262  
 An, Joon Geon 107, TP269, RP004, RP034  
 An, Taicheng WP189  
 An, Youn-Joo 579, WP124, WP170, WP171, WP172, RP203  
 Ananthasubramaniam, Bharath TP132  
 Anastas, Paul RP053  
 Anderle de Saylor, Marianna MP146  
 Andersen, Christian WP265, WP268  
 Anderson, Brian 447, 764, RP107, MP208  
 Anderson, Christopher 83  
 Anderson, Donald 590  
 Anderson, Erica MP078  
 Anderson, Jennifer MP232  
 Anderson, Julie 471  
 Anderson, Kim WP002, WP068, TP246, TP248  
 Anderson, Michael TP164  
 Anderson, Paul 130, MP139, TP150, TP169, MP220  
 Anderson, Todd 578, TP069, TP186, RP211  
 Andersson, Maria 35  
 Andrade, Natasha MP096  
 Ang, Choo Yaw TP103, TP014  
 Angenen, Largus 710, 711  
 Angradi, Theodore TP171  
 Ankley, Gerald 18, 25, 29, 122, 218, 223, 224, 380, 400, 558, 679, 769, RP090, TP134, MP138, MP149, RP151, MP151, MP152, RP222,  
 Annis, Mandy RP199  
 AnsaAsare, Osmund 371  
 Ansley, Melinda WP276  
 Anthony, Bonner 77, 78  
 Antkiewicz, Dagmara RP293  
 Antle, Patrick 646, RP001  
 Anton, Assumpcio 773, RP283  
 Aquilina, Noel 76  
 Aragon-Jose, Alejandra 728, WP053, TP094  
 Araujo, Vinicius 712  
 Arblaster, Jennifer 90  
 Arcaro, Kathleen 432  
 Arce-Corrales, Maria-Engracia MP089, MP090  
 Arciszewski, Tim RP259  
 Arens, Collin 566, WP036  
 Ares, Nichole RP241  
 Arey, Teresa 658  
 Arita, Norimasa TP141  
 Ariyoshi, Tadashi WP162  
 Arizono, Koji MP188  
 Armand, Stephane 57, TP028, RP041  
 Armbrust, Kevin 396  
 Armitage, James 399  
 Armstrong, Sarah 470, 578, WP042  
 Arndt, Devrah 39, 147, WP176  
 Arneson, Lisa 578  
 Arnold, Bob TP003  
 Arnold, Kathryn 708  
 Arnold, Mariah RP245  
 Arnold, Olivia RP172, WP285  
 Arnold, William 653  
 Arnot, Jon 204, 208, 642, 689  
 Arp, Hans Peter 91  
 Artal, Mariana 152  
 Arthur, Troy MP070  
 Arts, Gertie MP060, RP133  
 Arukwe, Augustine 565  
 Arzate Cardenas, Mario Alberto RP190  
 Asante, Kwadwo Ansong 175, 371, WP022  
 Ashley, Jeffrey 83, TP046, MP156  
 Ashpole, Sara WP061  
 Asselman, Jana 636  
 Atalay, Yasemin 395, 599, 602, TP022, MP180  
 Atlas, Ronald 214, TP015  
 Aufdenkampe, Anthony TP252  
 Auger, Kasie 432  
 Augspurger, Tom WP181, WP182, RP178, RP249  
 Aupinel, Pierrick 11  
 Aurand, Don 210, 392  
 Autry, Jamie MP142, MP162  
 Awata, Hiroshi 292  
 Awkerman, Jill 330, MP042  
 Ayre, Kimberly WP090  
 Azevedo, Ligia RP116  
 Azzam, Mohamad TP016, TP018  
 Azzonlina, Nicholas WP069  
 Babin, Patrick TP112  
 Baca, Sarah RP171  
 Bachman, Pamela 11  
 Backus, Sean 169, 196, 198, 476, 557, MP056, TP175  
 Bacon, Jamie 420, WP052  
 Badia-Fabregat, Marina 532  
 Baer, Kevin 222  
 Baer, Steven RP117  
 Baginska, Ewelina RP056  
 Baguley, Jeffery WP020  
 Bahamonde, Paulina TP130  
 Bahme, John 645  
 Baier-Anderson, Caroline TP240  
 Bailey, Frank 414, WP047, RP198  
 Bailey, Howard MP037  
 Bailey, Lisa MP197  
 Baily, Afonso 132, MP076  
 Baird, Patricia MP247  
 Baker, Christopher 219  
 Baker, Josh MP037  
 Baker, Katy 127  
 Baker, Mary 427  
 Baker, Stephanie 125, 674  
 Baker, Tyler MP177  
 Bakst, Murray WP279  
 Balbus, John 402  
 Balcom, Prentiss 35  
 Baldwin, Cheryl 687  
 Baldwin, David RP021  
 Baldwin, Susan RP093  
 Baldwin, William 623  
 Bammler, Theo TP059  
 Banerjee, Upasana 506  
 Bang, JiSu MP178, MP183  
 Banic, Cathy 475  
 Bank, Michael TP076  
 Banks, Cynthia MP226, WP249  
 Banman, Chris 120, MP161  
 Bao, Lian-Jun MP094  
 Baptistotte, Cecilia WP050  
 Bar-Ilan, Ofek WP169  
 Barbee, Gary WP096, RP276  
 Barber, Anglea TP201  
 Barber, David 314, 317, 342, MP078, RP295  
 Barber, Lynn MP217  
 Barber, Michelle 734  
 Barber, Timothy 529, 647  
 Barbero, Michelle RP198  
 Barcarolli, Indianara TP045, WP195, WP234  
 Barcelo, Damia 532  
 Bare, Jane 772  
 Barefoot, Aldos 724  
 Bargar, Tim MP078  
 Bargnesi, Keely TP075  
 Barham, Michael WP175  
 Barilone, Jessica 554  
 Barker, Joel 37  
 Barker, Natalie WP263  
 Barnes, Lajan 645  
 Barnett, Kelly RP278  
 Barrera Escorcia, Guadalupe MP173, MP190  
 Barret, Mailen TP073  
 Barrett, Timothy MP209  
 Barrick, Robert TP020, TP228  
 Barron, Mace 330, 741, MP042, MP048  
 Barrows, Elisabeth 160  
 Barry, Terence RP152  
 Barst, Ben RP281  
 Barst, Benjamin 32, TP230  
 Bartee, Margaret 731  
 Bartell, Steven WP091  
 Bartelt-Hunt, Shannon 24, 305, WP177  
 Barth, M Andrew RP136  
 Bartlett, Adrienne 616  
 Barton, Carlita RP153  
 Barton, Meredith 519  
 Bartos, Dana RP054  
 Bartoszek, Joseph WP120  
 Barzanji, Aras RP019  
 Basson, Rozell MP080  
 Basu, Niladri 336, 339, 378, 379, 381, 560, TP105, TP108, TP196, WP184, WP198  
 Batdorf, Carol RP074  
 Bates, Matthew 1, 423, 425, 429, 734, 786, WP094  
 Batista, Daniela TP048  
 Batt, Angela 254, 298, 301, 531, 638, TP004, TP170, TP171, TP260  
 Battaglin, William WP054  
 Baudrimont, Magalie 202  
 Bauer, Anthony WP034, WP044  
 Bauer, Megan RP091  
 Baumann, Zofia RP123  
 Baun, Anders 733  
 Bax, Danielle TP150  
 Baxter, Colden RP170  
 Baxter, Leilan RP157  
 Bay, Steven 451, 669, MP204  
 Baya, Pascale 36  
 Bayen, Stephane 158, 264, 356, TP063, TP212  
 Bazar, Matthew 385  
 Beahan, Erinn 421, MP038, WP223, WP226, RP272  
 Beaman, Joseph WP118  
 Bearden, Daniel 225, 763  
 Beaty, Lynne WP059  
 Beauchemin, Melissa 190  
 Beauparlant, David 241  
 Beaver, Earl RP117  
 Becher, Matthias 493  
 Beck, Brian TP095  
 Beck, Jonathan TP078, TP079  
 Becker, Matthew 735  
 Becker, Rick 123  
 Beckett, Kerrie MP175  
 Beckingham, Barbara 761, MP101  
 Beckner, Jeff 784  
 Beckwith, Bruce MP110  
 Bednar, Amy 736  
 Bednar, Anthony 703, TP097, TP098, WP159, WP166, TP201, WP271, RP287, RP291  
 Bednarz, James MP118  
 Beebe, Donald RP147  
 Beevers, Michael 10  
 Beganyi, Sarah WP238  
 Begley, Timothy TP033, TP034  
 Behniwal, Paramjit 369, TP136  
 Beirsto, Wendy RP122  
 Beischlag, Tim MP200  
 Beitel, Shawn RP181, RP187, RP204  
 Bejarano, Adriana 391, 427, TP031  
 Belanger, Scott 329, 335, 375, RP156  
 Belden, Jason 248, 249, 384, TP177, TP178, RP195, TP197  
 Bello, Nora WP253  
 Belosevic, Miodrag 241, 472, TP114

- Benaman, Jennifer 659, RP048  
 Bencic, David 29, 558, 679, RP090, TP134  
 Bendell, Leah MP247  
 Benedict, Lucas 554  
 Benedict, Lucille 554, RP017  
 Benfield, Mark 114  
 Beniash, Elia WP133  
 BenKinney, Marie TP027, RP033  
 Bennett, Deborah 71  
 Bennett, James RP209  
 Bennett, Richard WP079  
 Bennett, Samuel 142  
 Benotti, Mark 347, TP017, MP020  
 Benskin, Jonathan 255, 571, TP056, TP066, TP118, TP119  
 Benson, Bob TP004  
 Bentivegna, Carolyn 360, RP159  
 Bentley, Karin 123  
 Benvenuti, Mark TP266  
 Beramaschi, Brian 38  
 Bercovici, Sarah TP265, WP273  
 Berg, Craig WP103  
 Berg, Marlene 553  
 Berger, Carrie RP223  
 Berger, Urs TP057  
 Bergh, Kelli MP037  
 Bergknot, Magnus 68  
 Bergman, Ake MP230  
 Bergman, Harold 182, 299  
 Bergstedt, Roger TP013  
 Berli, Barbara 341  
 Bernardo, Matthew WP130  
 Bernatchez, Louis 202, 468  
 Bernhard, MaryJo TP085  
 Bernhardt, Emily 274  
 Bernhardt, Richard 383  
 Berninger, Jason RP030, TP215  
 Berntsen, Marc 94  
 Bertsch, Paul 327  
 Bess, Amanda MP203  
 Besser, John 228, 229, WP117, RP249  
 Bessie, Kathryn 576  
 Betts, Julia WP265, WP268  
 Bevilacqua, Jose WP243  
 Beyer, Richard TP059  
 Beyer, W. Nelson 510  
 Bezbaruah, Achintya RP080  
 Bhavsar, Satyendra TP054  
 Biales, Adam 452, 558  
 Bianchini, Adalto 53, 408, 507, MP040, TP045, TP180, WP195, WP202, WP203, WP207, WP219, WP234  
 Bicho, Rita 415  
 Bichraoui, Najet RP285  
 Bickerton, Greg 569  
 Bickham, John WP139  
 Bidleman, Terry 442, TP144  
 Bidwell, Joseph TP197  
 Biedenbach, James 696, RP033  
 Bielecki, Anthony 2  
 Bielefeld, Kristin MP031  
 Bielmyer, Gretchen WP197, WP216  
 Biever, Ronald MP147, MP164, MP165, TP256  
 Bignert, Anders TP077  
 Biksey, Thomas MP215  
 Billa, Nanditha TP071, TP208  
 Billock, Arlene RP173  
 Bireley, Richard 10  
 Biris, Alexandru Radu RP288  
 Birkholz, Detlef 174  
 Bisesi, Joseph WP149, WP153  
 Bishop, Christine 750  
 Bishop, Patricia 121  
 Black, Marsha 144, TP209  
 Blackburn, Mary TP078, TP079  
 Blacker, Ann 120  
 Blackmon, Sherry RP275  
 Blackwell, Brett 538, TP069  
 Blaine, Andrea 353  
 Blair, Benjamin 436  
 Blais, Jules 263, TP247  
 Blake, James 591, 693  
 Blake, Lindsey WP055  
 Blakeley-Smith, Mathew WP281  
 Blalock, Bonnie 559, WP176  
 Blanchard, Pierrette 475  
 Blanchfield, Paul WP082, RP253  
 Blankenship, Alan WP266  
 Blanksma, Chad WP055, MP149  
 Blaquez, Paqui 532  
 Blazer, Vicki 515  
 Blechinger, Scott 149  
 Blewett, Tamzin MP046, MP047  
 Blickley, T Michelle WP167  
 Bluhm, Kerstin 711, 713  
 Blum, Arlene 166  
 Blusztajn, Jerzy 652  
 Bock, Michael 529, 647  
 Bockelie, Adam 428  
 Boddu, Veera 736  
 Boden, Adrienne MP055  
 Bodensteiner, Scott 280  
 Bodinier, Charlotte 392.5  
 Boehm, Leonard TP088  
 Boehm, Paul 213, 214, TP020, TP159, TP228  
 Boehme, Susan RP024  
 Boehnker, Dave MP135  
 Boer, Jacob MP017, WP075  
 Boesch, Donald 111  
 Boethling, Robert 587, TP240  
 Boettcher, Anne TP026  
 Bohentin, Brett 183  
 Boireau, Veronique RP056  
 Boivin, Thomas 182  
 Bolado-Martinez, Enrique MP089  
 Bolgrien, Dave TP171  
 Bolotin, Jakov 460  
 Bols, Niels 195  
 Bondi-Marschner, Laila 779  
 Bone, Audrey 46, RP013, WP161, TP202  
 Bonnell, Mark 150, 209, 328, TP085  
 Bonnevie, Nancy 190, TP150  
 Bonventre, Josephine TP131  
 Booc, Frank MP073  
 Boone, Scott TP004, TP005  
 Boone, Tripp TP005  
 Boorse, Howard TP205  
 Booth, Pieter MP084  
 Boothman, Warren 161  
 Borchers, Christoph 220  
 Borgert, Chris 123, 332  
 Borgert, Christopher 118  
 Borgos, Sven Even 313  
 Borkman, David 590, 697  
 Born, Erik TP052  
 Bornstein, Jason 448, WP077  
 Boroujerdi, Azeuz 225  
 Borrok, David RP225  
 Borski, Jeffrey RP134  
 Borton, Dennis RP166  
 Bortsie-Aryee, Nana RP192  
 Bosker, Thijs MP045, MP047, MP072  
 Bossart, Gregory 235  
 Bothner, Michael 698  
 Botta, Clarice WP199  
 Boughton, Raoul 247  
 Bouland, Andrew WP251  
 Bouldin, Jennifer 624, WP123, RP267  
 Bourgault, Guillaume RP119  
 Bourne, Amy 5  
 Bousquet, Stephen 8  
 Bouwer, Edward 55, TP195  
 Bouwmeester, Hans 266  
 Bowers, Wayne MP108  
 Boxall, Alistair 402, 430, 433, 708 WP154  
 Boyd, Robert 703, WP263  
 Boyd, William 692  
 Boyd, Windy 133  
 Brack, Werner 453, WP072, WP073, WP074  
 Bradley, Kimberly WP128  
 Bradley, Michael MP136, TP167  
 Bradley, Patrick MP035, MP180, MP201  
 Brady, Steven WP053  
 Bragg, Leslie WP034, MP047, TP250, TP251  
 Brain, Richard 491, WP091, RP157, RP252  
 Brakstad, Odd 216  
 Brame, Jon TP098  
 Bramley, Yvonne 491  
 Brammell, Ben RP183, RP278  
 Brandenberger, Jill 102, MP110  
 Brandimarte, Ana WP199  
 Brandon, April MP202, WP283  
 Brandsma, Sicco 19, MP017  
 Brandvik, Per TP019  
 Brasfield, Sandra 404, MP226, RP250, WP271, WP276  
 Braun, Chris 439  
 Braunbeck, Thomas 329, 333  
 Braunig, Jennifer 333  
 Breedveld, Gijis 86, 760  
 Breen, Michael 679  
 Breen, Miyuki 679  
 Bremner, Kieren MP080  
 Brenda, Sanders MP185  
 Brenemann, Curt 544  
 Brenkart, Karl 763  
 Brennan, Amanda 41  
 Brenner, Richard 193  
 Breton, Roger MP169, MP184  
 Brewton, Rachel RP261  
 Brice, Ken TP175  
 Bridges, Todd RP086  
 Briggs, Dean MP087  
 Brimacombe, Corina 555  
 Brindle, Ian MP008  
 Bringolf, Robert 21, WP097, RP105  
 Brinkman, Fiona RP037  
 Brinkmann, Markus 711  
 Brinson, Kenny 183  
 Brisebois, A 93  
 Brisset, Jean RP233  
 Brittain, Claire 9  
 Brix, Kevin 232, 673, WP131, WP229  
 Broadway, Bryanna WP132  
 Brock, Jonathan WP216  
 Broder, Michael TP239  
 Brody, Lyle 74, 304, WP013  
 Brook, Jeff MP155  
 Brooks, Bryan 430, RP030, RP053, WP095, TP210, TP215  
 Brooks, Majorie 403  
 Broussard, Christine 234 WP104, TP116, TP117  
 Brown, Ashli 396  
 Brown, Carl WP190  
 Brown, Daniel 564  
 Brown, John 210, 211, 605, TP027, RP033  
 Brown, Lisa 616  
 Brown, Lorraine , RP037  
 Brown, Mitra 477  
 Brown, Murray 509  
 Brown, R 195, 196, 199, MP070, WP077  
 Brown, Stephen 197  
 Brown, Trevor 208, 642, 689  
 Brownawell, Bruce 657, 694, RP040  
 Browning, David 397  
 Browning, Zac 10  
 Brozinski, Jenny-Maria RP044, TP062  
 Bruce, Lyle 210  
 Bruce, Mark 58  
 Brueggen, Teresa RP267  
 Brugger, Kristin WP257  
 Brumbaugh, William 228, 229, RP050, WP107, WP117, WP224, MP240, MP241  
 Brun, Olivier 322  
 Bruno, Maribel RP078  
 Bruton, Thomas 270  
 Bryant, Douglas 782  
 Bryant, Wade RP033  
 Buchwalter, David 505, 508, MP041  
 Buck, Kenton WP277  
 Buck, Loren 383  
 Buck, Robert 250, TP055, TP057  
 Buckler, Justin WP115  
 Budinsky, Robert 722  
 Budzinski, Helene 202  
 Buechi, Stephen TP102  
 Bugbee, Bruce 549  
 Buhle, Eric RP021  
 Bulbule, Kesav 457  
 Bulle, Cecile 706, 775  
 Bulloch, Daryl 461  
 Bunch, Aubrey WP066  
 Burbank, Teresa 439  
 Burdge, Russell RP136  
 Bureau, Ronan MP001  
 Burger, Greg WP259  
 Burgess, Emily 116  
 Burgess, Jennifer TP242, TP266  
 Burgess, Neil TP194,  
 Burgess, Oliver MP043, RP212  
 Burgess, Robert 145, 261, 446, 452, 757, WP020, TP227  
 Burgoon, Lyle RP038  
 Burkhard, Lawrence , TP071, TP208  
 Burklew, Caitlin 521  
 Bursian, Steven 27, 514, 719, WP114, WP253  
 Burton, Allen 407, WP018, RP235  
 Buser, Michael 538  
 Bushey, Joseph 728, RP020, WP053, TP094  
 Bushnaf, Khaled 92  
 Busquet, Francois 329  
 Bussolaro, Daniel RP009  
 Butcher, Matt MP225  
 Butler, Eric RP003, RP035  
 Butt, Craig 454, 459  
 Buzby, Mary TP169  
 Byer, Jonathan 195, 196, 197, 199, 200, MP070  
 Byl, Tom RP019, MP102, MP106, MP136  
 Byrne, Christian TP005  
 Byrne, Jon 225  
 Byron, Earl 670, MP212  
 Bystedt, Melissa WP121, RP131  
 Byttingvik, Jenny WP075  
 Bytwerk, David 501  
 C C F Nocelli, Roberta 11  
 Cach-Perez, Eric 258, WP246  
 Caetano, Miguel MP027, MP028, TP253  
 Cagnin, Renata WP203  
 Cai, Minghong TP040  
 Cai, QingSong 538  
 Cains, Mariana RP269  
 Calafat, Antonia , MP119  
 Caldwell, Daniel 126, 130, 430  
 Calfee, Robin TP176, WP223, WP226, WP230, RP272  
 Callard, Gloria 54  
 Camacho, Lucy 350  
 Cameron, Rory WP038  
 Camilleri, Sandra WP110  
 Caminal, Gloria 532  
 Campbell, Dave RP166  
 Campbell, Peter 202, 409, 411, 468, RP031, WP213, WP217, WP218, WP231  
 Campbell, Todd WP062  
 Campe, Lisa 297  
 Canady, Richard 269  
 Canario, Joao 31, 289, MP027, MP028  
 Canas, Jaclyn TP182, TP186, TP189  
 Candrl, James WP115  
 Cangialosi, Maria 565  
 Cantarella, Barbara WP203  
 Canton, Steven 275, 276, 662, 674, RP108  
 Cantor, Robin MP167  
 Cantwell, Mark 261, 446, WP020, TP227  
 Capdevielle, Marie 529  
 Capel, Paul 444, 445, TP149  
 Capolupo, Casey WP204  
 Carbonaro, Richard 230, 597, 599, 602  
 Carbone, John RP139  
 Card, Marcella 354  
 Cardamone, Andrew WP247  
 Cardinale, Bradley 142  
 Cardon, Mary 223  
 Cardozo, Janaina TP180  
 Carey, Andrea 201, MP069  
 Carignan, Courtney 179, MP127  
 Carini, Franca 500  
 Carlson, Jenna TP193



- Carlson, Jules WP004  
Carmichael, Catherine 212  
Carmichael, Gregory 539  
Carmichael, Ruth TP026  
Caron-Beaudoin, Elyse MP016  
Carr, Deborah 578  
Carr, Gregory 329  
Carr, R Scott 696  
Carragher, Peter 217, TP035  
Carrasco Navarro, Victor 606  
Carraway, Elizabeth 140, 162, 308  
Carrere, Helene TP073  
Carretero, Miguel 415  
Carrick, Toni TP002  
Carrier, Latonya RP247  
Carriker, Neil 96, MP177  
Carriquiriborde, Pedro RP032, TP133, RP263  
Carrizo, Daniel 63  
Carro, Tiffany WP186  
Carroll, David 40  
Carroll, Leslie 227  
Carter, Laura WP154  
Carvalho Rodrigues, Sandra TP045  
Carvan, Michael 379, 381, WP103, TP105  
Cary, Tawnya 236  
Casey, Ryan RP022, RP025, RP125, RP128, WP237  
Casper, Andrew RP250  
Caspers, Norbert TP001  
Cassada, David 159  
Cassani, Stefano 683  
Casselman, John 195, 197, 199  
Cassidy, Brianna TP039  
Cassimiro, Laene RP076  
Cassone, Cristina 338  
Castle, James RP145, RP147, RP164  
Castrodale, Louisa 495  
Catching, Clayton WP180  
Catchpoole, Heather 273  
Catoi, Cornel RP288  
Caumette, Guilhem WP196  
Cavallin, Jenna 224, MP149, RP151  
Cavicchioli-Azevedo, Vinicius 408  
Cecillon, Sebastien TP044  
Cekic, Nevena MP108  
Celo, Valbona MP117  
Certo, Matt MP225  
Cesario, Rute MP028  
Cessna, Allan 665, RP045  
Chabot-Giguere, Bernice MP014  
Chadwick, Bart MP134, MP135  
Chakraborty, Paromita WP023, TP039, TP146  
Challis, Jonathan WP004  
Chalmers, Ben MP037  
Chambers, Patricia MP144  
Chambers, R 52, 467  
Chambliss, C TP210  
Chambliss, Kevin TP215  
Champlin, Denise 135, 462, 630  
Champoux, Louise MP015  
Chan, Katherine WP228  
Chan, KokYeng WP214  
Chandalia, Jui 438  
Chandler, David 493  
Chandler, G 145  
Chang, Feng 445  
Chang, Feng-Chih MP054  
Chang, Kwang-Hyeon MP124  
Chanov, Michael 281  
Chao, Jingbo 268  
Chapman, Peter 403, 607, RP101, RP137  
Chappell, Marc 736  
Chappell, Mark 734, WP249  
Chappell, P MP239  
Chappell, Pornsawan TP097, MP237  
Chard, Julie 76, 548, 549  
Chariton, Anthony WP020  
Charland, Jean-Pierre 475  
Charles, Raphael 773  
Charnock, John RP124  
Charpentier, Michael 261  
Charters, David 82  
Chau, Jessica RP020  
Chaudhuri, SriR. MP155  
Chauzat, Marie-Pierre 10  
Chavez, Nita 357  
Checkai, Ronald 386, TP099, TP104  
Chen, Aimin 456  
Chen, Celia 146, 465  
Chen, Chien-Min RP175  
Chen, Chunli 105  
Chen, Da 167, MP012  
Chen, Fangfang TP212  
Chen, Fanghui 659  
Chen, Jian 39  
Chen, Jingwen 684  
Chen, Kai Loon 349  
Chen, Mei RP039  
Chen, Qu RP007  
Chen, Robert RP051  
Chen, Sha MP005, RP114  
Chen, Tony 555  
Chen, Wenlin MP186  
Chen, Xuedong RP215  
Chen, Yu WP261, WP270, WP279  
Chen, Zhongzhi WP213  
Cheng, He TP050  
Cherchi, Carla 625  
Cherchia, Elizabeth WP148  
Cherry, Donald RP150  
Chew, Bill WP126  
Chiavelli, Deborah RP048, TP083  
Chiewchanchai, Mukrekha WP188  
Chin, Diana 364, MP220  
Chin, Yu-Ping 354, 545, RP049, TP238, TP267  
Chinalia, Fabio 712  
Chitwood, Rob WP119  
Chiu, Suzanne 338  
Chiu, Yuh-Wen RP175  
Cho, Jaeweon 300  
Cho, Kijong RP059, RP169, RP176, WP280, WP284  
Cho, Yeo-Myoung 85  
Choate, James WP123  
Choi, Cheol Young WP138  
Choi, Jinhee 325, RP078, WP142, WP143, RP290  
Choi, Kyungho 430, 639, TP151, WP138, WP157, RP218, RP246, RP260  
Choi, Sung-Deuk 458  
Choi, Yeyong TP151  
Choi, Yongju 85  
Choongo, Kennedy TP213  
Chou, Monidarin TP063  
Chouinard, Naomi 554  
Chowdhury, Jasim WP210  
Christ, Mark WP257, WP275  
Christian, Alan RP052  
Christiansen, Karen WP200  
Christie, Andrew 222  
Chu, Eric WP094  
Chu, Erik 428  
Chu, Ih MP108  
Chu, Shaogang 167, MP012  
Chumchal, Matthew 32, MP092, TP230, RP273  
Chung, Gi-Woong WP143  
Chung, Katy 394  
Chung, YunDoo RP290  
Cibor, Adrienne 730, RP236  
Cieniawski, Scott 193, 194  
Cincinelli, Alessandra 537, RP043  
Cirillo, Piera 455  
Citra, Mario TP240  
Clarence, Stacey 316  
Clark, Bryan 464, 564  
Clark, Candace WP160  
Clark, James 392  
Clark, Kathryn TP001  
Clark, Stephen 614  
Clarke, Joan 188  
Clarke, Peter MP017  
Clarkson, Jacquelyn 781  
Claude, Jeremy 116  
Clayden, Meredith 33  
Claytor, Carrie 125, 674, WP128  
Cleland, Gareth TP242  
Clements, William 401, 663, 668  
Clichici, Simona RP288  
Cline, George WP057  
Clock-Rust, Mary 575, MP171  
Clough, Stephen 601  
Clouzot, Ludiwine WP082  
Coady, Katherine 117, MP163  
Coakley, Jonathan MP126  
Coats, Joel MP232  
Cobb, George 538, RP271  
Cochran, Michele 443, RP043  
Codling, Garry 65, 68  
Coe, Tobias MP081  
Coelho, Gina 210, 392  
Cohen, Stuart 645  
Cohn, Barbara 455  
Coiro, Laura 161  
Colbourne, John 465, 635, 636, TP187  
Cole, Jennifer 416, WP049  
Cole-Neal, Cavelle RP143  
Coleman, Ben WP161  
Coleman, Deborah RP055  
Coleman, Jessica WP166, WP271, WP276, RP291  
Coleman, Victoria 273  
Collette, Timothy 29, 223, 765, 769, MP138  
Colli-Dula, Reyna C 317, 727  
Collier, Zachary 5, 422, 786, WP094  
Collins, Chris 89  
Collins, Daniel 628  
Collins, Joshua MP109  
Collins, Leonard 464  
Colman, Ben 46, TP202  
Colombo, Fabio 671  
Colton, Jenee 729, MP234  
Colvin, Marianne RP155, WP204  
Colvin, Molly 730  
Conder, Jason 671, TP057, RP069  
Condon, Anne 517, WP111  
Conerly, Octavia TP004  
Cong, Lin TP023  
Conklin, Julie 783  
Conley, Justin 508  
Connelly, Michael 290  
Connery, Bruce WP255  
Connolly, John 659, RP048, TP083  
Connon, Richard 318  
Connor, Kevin TP155  
Connors, Kristin TP210, TP215  
Conoan, Nicholas 24  
Conolly, Rory 679  
Conquest, Loveday RP018  
Conrow, Roxanne 751, 752  
Conry, Tom TP215  
Consbrock, Rebecca MP039, WP224  
Constantine, Lisa 124, WP146, RP188  
Conte, Brian 181  
Conti, Edward WP244  
Convertino, Matteo 425, WP078, RP086, RP089  
Cook, Diana RP166  
Cook, Linda 211, 214, TP020, TP228  
Cook, Michelle WP254  
Cooke, Dan MP036  
Coombe, Vyvyan 129  
Cooper, Cynthia 160  
Cooper, Ellen 166, 179, 464, MP010  
Cooper, Keith 360, TP111, TP113, TP131, RP165, WP201  
Cooper, Naomi RP195  
Cooper, Patricia 250, TP055  
Cooper, Rebecca MP118  
Cooper, Scott WP250  
Cooperstein, Sharon 687  
Cordeiro, Christopher MP156  
Cordeiro, Tamires RP076  
Cormier, Susan 664, 666  
Cornelissen, Gerard 86, 760  
Corrales, Jone TP026  
Corsi, Steven 153  
Corvi, Margaret TP107  
Cosgrove, John TP056, TP066, TP119  
Costa, Emily Jane 143  
Costanza, Jed TP240  
Costello, David 407, RP235  
Cotsifas, Jeffrey 614  
Cotta, Michael 710  
Cotter, Kellie 54  
Coty, Janna MP030  
Couillard, Catherine 195, 200, 202, MP075  
Coupe, Richard 444, 445, TP149  
Cousins, Ian 399, TP057  
Coutermarsh, Bonita MP071  
Couture, Patrice 202, 468  
Covaci, Adrian 176, 178, 374, RP065, MP126, TP142, TP143  
Coveney, Michael 751  
Cowan-Ellsberry, Christina WP019, TP085  
Cowen, Nicola WP259  
Cox, Stephen 187, TP023, TP189, RP242  
Crago, Jordan 39, 436, MP148  
Craig, Peter 682  
Crane, Cynthia 484  
Crane, Michael WP136, TP170, TP171  
Cranor, Walter 344, 441  
Crawford, Sarah RP186  
Cremazy, Anne 411  
Cresmilleux, Bruno MP001  
Crespo, Diana RP263  
Crimmins, Bernard 164, 165, MP054, MP058, RP064  
Cristol, Dan 517, TP193, WP277  
Cristol, Daniel 400, 512, WP111, TP172, WP251  
Crk, Tanja 575, MP171  
Crocker, Joe MP195  
Crocker, Tracey MP203  
Crofton, Kevin 22  
Crocket, Emma 57, TP028, RP041  
Croskrey, Jennifer 61, TP233  
Cross, Wyatt RP170  
Crossley, Alison 286  
Crouch, Caitlin 405  
Crum, Doug 27, 338, 518, 719  
Crumrine, Patrick 247  
Cruz-Mirazo, Enrique MP090  
Cryderman, Diana WP198  
Csiszar, Susan 556, 557, WP180  
Cuenca Navarro, Ana 317  
Cuissart, Bertrand MP001  
Culp, Joseph 474, 665  
Cumberland, Howard MP202  
Cunningham, Candice TP138  
Cunningham, Jessie 412  
Cunningham, Mark WP283  
Cunningham, Virginia TP169  
Cura, Jerome MP193  
Currie, Rebecca 117, MP163  
Currie, Suzanne MP047  
Curtis, Emmet WP121, RP131  
Cusaac, J Patrick 414, WP047  
Cusack, Paul MP017  
Cushing, Kerry 160  
Cutler, Geoffrey MP070  
Cutter, Jessica 470  
Cwiertny, David RP274  
Czerepak, Erica WP122  
D'Aco, Vincent TP169  
D'Onofrio, Elisa 683  
D'Silva, Joshua 506  
Dabek-Zlotorzynska, Ewa MP117  
Daessle Heuser, Luis Walter TP264  
Daggupaty, Sam 556, 557  
Dahle, Jessica WP010  
Dahlen, Deirdre 591, 693, 695  
Daicoviciu, Doina RP288  
Dailey, Katie TP128  
Dailey, Meghan TP026  
Daley, Jennifer TP086  
Daling, Per 216, TP021  
Daling, Per S TP019

- Dalziel, John MP026  
 Damian, Kirchmajer RP217  
 Dan, Johnson RP215  
 Dang, Viet WP010, MP104  
 Dangerfield, Neil 97  
 Daniel, Farrar MP240  
 Danielsson, Sara TP077  
 Das, Kaberi TP053  
 Das, Pranab 627  
 Das, Siba TP107  
 Daso, Adegbenro WP005, WP006  
 David, Breshears 4  
 David, Frank RP066  
 Davies, Jo RP133  
 Davis, Elizabeth 546  
 de Jonge, Lis MP121  
 De La Paz, Nayeli RP214  
 De La Rosa, Vanessa WP101  
 de la Rosa Perez, Alejandro RP268  
 De La Torre-Roche, Roberto TP002  
 De Laender, Frederik WP140  
 de Lima, Rodrigo 155  
 De Schamphelaere, Karel A.C. 401, 636  
 De Silva, Amila 169, 171, 198, 251, 252, 255, TP058, TP087  
 de Solia, Shane 169  
 de Voogt, Pim TP057  
 De Zwart, Dick 667  
 Deacon, Samantha RP087, RP149  
 Dean, Karen 519, WP256, WP282  
 DeAngelis, Donald RP161  
 Deardorff, Thomas MP222, WP252  
 DeBano, Sandra RP142  
 Decea, Nicoleta RP288  
 Deconinck, Dieter 636  
 Decourtye, Axel 11  
 Dee, Justin 702  
 DeForest, David 226, 232, 673  
 DeFranco, Emily 456  
 Degitz, Sigmund 26, WP055  
 Degner, Ethan MP048  
 Degnes, Kristin 313, 767, RP094  
 DeHate, Robin 181, 184  
 DeJager, Lowri TP033, TP034  
 DeJong, Grant 275, 276  
 Deki, Noriko WP220  
 Del Vento, Sabino 65, 68  
 Delach, Diana 98, 307, WP010, WP027, WP104  
 Delecki, Anissa WP269  
 Deleebeek, Nele RP227  
 DeLeon, Sara 337  
 Delfosse, Thomas WP125  
 Delgenes, Nadine TP073  
 Delistraty, Damon TP174  
 DeLorenzo, Marie 144, 394  
 Delorme, Peter MP063  
 Delov, Vera 334  
 DeMott, Robert TP157  
 Deng, Dong-Fang WP060  
 Deng, Shuguang 350  
 Denslow, Nancy 20, 221, 314, 317, 342, 382, 723, 727, RP015, MP078, WP102, TP123, WP174  
 Denton, Debra RP106, RP262  
 DePauw, Edwin MP150  
 DeQuattro, Zachary RP152  
 Desai, Malan Manish 264  
 Desbiens, Isabelle RP006, RP009  
 Deschenes, Louise 706, 775, RP116  
 Desforgues, Melissa WP040, WP041  
 DeShields, Bridgette 190, MP224  
 Deshler, Tad 185  
 DeSmet, Amy RP153  
 DeSorbo, Christopher WP255  
 Dettenmaier, Erik MP155  
 Devanathan, Gnanasekaran TP074  
 Devanathan, Gnanasekaran 175  
 Devanathan, Gnanasekaran 371  
 Devanathan, Gnanasekaran 457  
 Devellis, Stephen TP256  
 Devries, Rebecca MP036, WP241  
 DeWall, Jeanne 370  
 Dewall, Jeanne 551  
 Dhondt, Andre 337  
 Di Benedetto, Ana Paula RP072, MP157  
 di Cenzo, Peter 473, 474  
 Di Giulio, Richard 274, 464, 564, RP245  
 Di Toro, Dominic 387, 388, 599, 602, MP002, TP084, TP099, TP100  
 Diamond, Jerome 637, RP106, RP262  
 Diamond, Miriam 310, 555, 556, 557, MP155, WP179, WP180, WP232, RP244  
 Diamond, Rachael 603, WP219  
 Diamond, Stephen 41  
 Diaz, Dale 396  
 Diaz, Robert 592  
 Diaz-Cruz, Silvia 532  
 DiazJaramillo, Mauricio 505  
 Dickerson, Virginia 377  
 Dickhut, Rebecca 443, RP043  
 Dickinson, Gary WP133  
 Diderich, Robert 28  
 Dien, Bruce 710  
 Dietrich, Kim 456  
 Dietz, Rune TP052  
 Diez-Ortiz, Maria 323  
 Diggs, Nora 70, 550  
 DiGiulio, Richard 46, TP125  
 Dillihay, Elliot 692  
 Dillon, Frank MP134, MP135, MP212  
 Dimitrov, Sabcho 208  
 Dimond, Stephen TP001  
 Din, Mohamed 472, 568  
 Ding, Yuping WP065, TP259  
 Dinius, Blake RP132  
 Dinter, Axel 11  
 Dionne, Rayann WP122  
 DiPinto, Lisa 393  
 Dirinck, Eveline 178, 374, TP143  
 Dirtu, Alin 176, MP126, TP142, TP143, RP065  
 DIRTU, Alin 178, 374  
 DiStefano, Robert WP107, WP117  
 Dixon, D WP034  
 Dixon, Kenneth WP106  
 Dobbs, Michael 120, 724  
 Dodd, Matt RP122  
 Dodder, Nathan RP068  
 Dodson, Robin 74, WP013  
 Doering, Jonathon RP181, RP187  
 Doering, Laszlo RP135  
 Doherty, Anne 657, RP040  
 Dohmen, Peter RP133  
 Dolan, Benjamin WP100  
 Dominguez, Gustavo 20, RP013  
 Dominy, Warren WP060  
 Domoradzki, Jeanne TP085  
 Doney, Gregg TP025  
 Dong, Yan WP200  
 Dongsheng, Chen 101  
 Donnelly, Kirby WP139  
 Donohue, Keri TP014, RP081, RP286  
 Donohue, Maura TP004  
 Donovan, Ellen MP146  
 Doody, Paul 190  
 Doperalski, Nicholas 342  
 Dorais, Martine RP283  
 Doren, Douglas MP002  
 Dorn, Philip 335  
 Dornbos, Peter 336  
 Dorrington, Tarquin 132  
 Dorsman, Edith MP060  
 Doucette, William 76, 548, 549, MP155  
 Doug, Clarke 188  
 Douglas, Helen 77, 78  
 Douwes, Jeroen MP126  
 Down, Tiffany 575, MP171  
 Doyle, Meghan MP072  
 Drevnick, Paul TP194, TP230  
 Driscoll, Charles 38, TP200  
 Drouillard, Ken 207, TP086, TP263  
 Drzewicz, Przemyslaw WP043  
 Du, Bowen TP210, TP215  
 Du, Juan RP221  
 Du, Songyan 718  
 Dubansky, Benjamin 392.5, WP227  
 Dube, Monique WP221  
 Ducrot, Virginie MP150  
 Duering, Rolf-Alexander TP088  
 Duffy, Tara 523  
 Dufour, Sylvie 202  
 Dugo, Giacomo 565  
 Duke, Clifford 740  
 Dunagan, Sarah 304  
 Dunlevy, Peter 511  
 Dupont, Ryan 549  
 Durda, Judi 403  
 Durell, Gregory MP020  
 Durham, Jeremy 99, MP035, RP194  
 Durhan, Elizabeth 223, 224, 769, MP138, MP149, RP151, MP151, MP152, RP222  
 Durrant, Lucia 154  
 Dvarskas, Anthony 427, WP081  
 Dyck, Nancy WP260  
 Dyer, Scott 103, 527, 643, WP015, RP139  
 Dyke, Gary MP134  
 Dynes, James 44  
 Dzioba, Marisa MP071  
**E** Eagle, Sarah 177  
 Earley, Patrick RP155, WP204  
 Eastling, Paul 552  
 Ebbert, Steve 511  
 Ebert, Ellen 80  
 Ebinghaus, Ralf 65, MP018, TP040, TP041  
 Ebke, Peter RP135  
 Echols, Brandi RP150  
 Echols, Kathy 70, 513, 550, RP050  
 Eck, William 585  
 Edelmann, Eva 89  
 Edgington, Aaron 376, RP081, RP286  
 Edmonds, Samuel 31, RP130  
 Edson, Jeffrey 750  
 Edwards, Donn RP206  
 Edwards, Melanie 594, TP015, RP033  
 Edwards, Peter 491  
 Edwards, Phillip RP239  
 Edwards, Stephen 29, RP038  
 Eede, Nele 176, MP126  
 Eek, Espen 86, 760  
 Eguchi, Akifumi 457, WP026, TP074  
 Eickhoff, Curtis TP082  
 Eitzer, Brian MP067  
 Ekman, Drew 29, 223, 765, 769, MP138  
 Eldin, Mohamed 471  
 Elfs, Cristiane 51  
 Elie, Pierre 202  
 Elizalde Ramirez, Laura MP173, RP279  
 Ellick, Rachel WP253  
 Elliott, James TP075  
 Elliott, John 67, 747, 748, 750, MP200, WP272  
 Elliott, Kyle 67  
 Ellis, David 656  
 Ellis, Jamie 11  
 Ellis, Thomas WP035  
 Elmquist, Marie 760  
 Elorriaga, Yanina RP032  
 Elphick, James MP037, TP127, TP129  
 Elrod-Erickson, Matthew MP082, RP191  
 Elskus, Adria 246  
 Ely, Patrick 21  
 Embry, Irucka MP106  
 Embry, Michelle 335, 375, TP085  
 Emma, Schymanski 453, WP073  
 Emmerton, Craig 37  
 Emsbo-Mattlingly, Stephen 73, RP002  
 Eng, Margaret WP272  
 Engelhardt, Heidi TP251  
 Eom, HyeonJeong RP290  
 Epp, Jessica MP056  
 Erdem, Ayca WP158  
 Erdman, Nicholas 552  
 Erhardt, Susan TP085  
 Erickson, Matthew WP267  
 Erickson, Richard 187  
 Erickson, Russell 502, RP097, RP103  
 Ericson, Jon 129  
 Erler, Steffen WP155, RP193  
 Erwin, Kyle TP125  
 Esbaugh, Andrew WP131, WP229  
 Escalante, Priscilla 234  
 Escalon, Lynn 224, RP090, TP097, TP112, TP134  
 Espejel Pina, Laura MP190, RP279  
 Espejel Trejo, Jose RP279  
 Espindola, Evaldo WP199  
 Estes, Tammara MP169, MP186  
 Etre, Neal 427  
 Etterson, Matthew WP079  
 Eustis, Soren 653  
 Evans, Marlene MP030, MP031  
 Evers, David 512, 517, TP025, MP029, WP111, RP130  
 Evers, David C TP105  
 Evgenidou, Angeliki 693  
**F** Faber, Tom 298  
 Factor-Litvak, Pam 455  
 Fadel, James MP159  
 Fahlman, Brian 470  
 Fair, Patricia 235  
 Fairbrother, Anne WP018, MP172, MP207  
 Fairchild, James WP107, WP117, RP143  
 Faksness, Liv-Guri TP021  
 Falciani, Francesco RP038  
 Falconer, Renee 443  
 Falso, Paul 418  
 Fan, Ming WP125  
 Fan, Ruifang 369, TP139  
 Fang, Hansun WP189  
 Fang, Xiefan MP073  
 Fantke, Peter 773  
 Farenhorst, Annemieke RP202  
 Farin, Frederico TP059  
 Farley, Kevin 230, 597  
 Farmahin, Reza 27, 518, 719  
 Farr, James 391  
 Farrar, Daniel MP238, MP239  
 Farrar, JD MP241  
 Farrar, John MP237  
 Farrelly, Eamonn 491  
 Farruggia, Frank 575, MP171  
 Fatoki, Olalekan WP005, WP006, RP243  
 Fauser, Patrik 180  
 Faust, Derek TP023  
 Faustino, Augusto 415  
 Fava, James RP117  
 Fay, Cash TP224  
 Fay, Kellie 206  
 Fedorak, Phillip WP037, RP141  
 Feiler, Ute 333  
 Feist, Blake RP021  
 Fellers, Gary WP054  
 Feltz, Kevin 70, 550, RP050  
 Feng, Yong-Lai WP191  
 Fennell, Donna 718  
 Fenske, Martina 334  
 Fenske, Richard 402  
 Ferguson, Lee 145, 153, 218, 394, 694, RP013, MP024, MP111  
 Ferguson, P 166, 732  
 Ferguson, P Lee 271  
 Fernandes, Luiz WP203  
 Fernandez, Joseph 502  
 Fernandez, Loretta 450  
 Fernandez Rendon, Carlos MP190  
 Ferrario, Joseph TP005  
 Ferreira, Claudia MP091  
 Ferreira, Roger MP076  
 Ferrell, Joshua RP278  
 Feswick, April RP295  
 Ficko, Sarah MP132  
 Fidler, Stephanie WP109  
 Field, Jason 4  
 Field, Jay 485  
 Field, Jeffrey TP241  
 Field-Juma, Alison 432  
 Figueiredo, Kaisa 59, WP067  
 Fikslin, Thomas TP046  
 Filip, Adriana RP288  
 Fillmann, Gilberto TP043  
 Findlay, Dave WP082

## AUTHOR INDEX

- Firkus, Tyler TP109, TP110  
 Firmage, Dave MP155  
 Fischer, David 9, 16, MP063, MP068  
 Fischer, Katharina TP194  
 Fischer, Ralf 9  
 Fish, Lucy MP183  
 Fisher, Daniel 17, RP025  
 Fisher, Jeffrey WP064, MP113, TP140  
 Fisher, Nicholas RP123  
 Fisher, Robert RP166  
 Fisk, Robert RP018  
 Fitzgerald, Patrick 657  
 Fitzgerald, Scott 514, WP114, WP253  
 Fitzsimmons, Patrick 206, TP210  
 Fjeld, Eirik TP077  
 Flanagan, Joseph 562  
 Flanders, John 95, MP109, WP192  
 Fleischer, Mark TP256  
 Fleming, Carrie MP236  
 Fleming, Margaret WP017  
 Fleming, Tyler MP156  
 Fletcher, Tim 484  
 Flewelling, Samuel 648  
 Flick, Robert 558, 638, WP136  
 Flight, Patrick 135  
 Flinders, Camille RP166  
 Flint, Suzanne 432  
 Flocken, Katie RP236  
 Floriano, Nubia TP048  
 Flynn, Robert WP267, WP274  
 Fogg, Andrea 483, 785, MP130, WP260  
 Folland, William MP123, MP201  
 Folle, Solomon MP184  
 Folmer, Henry 396  
 Folsom, Patrick 250, TP055  
 Fontes, Grazielle 155  
 Foran, Christy 422, 425  
 Forbes, Valery 203, WP085, WP086  
 Foreman, William 439, 444, 445, TP149  
 Forlin, Lars 48  
 Forrest, Casey 659, RP048  
 Forrester, Harrison 441  
 Forsberg, Norman TP248  
 Forsgren, Kristy RP274  
 Fort, Chelsea 420, WP052, MP142  
 Fort, Douglas 420, WP052, MP142, MP162  
 Fort, Hayley 420, MP142  
 Fortin, Claude 409, 411, RP031, WP213, WP231  
 Foster, Karen 263  
 Foster, Kimberly TP268  
 Foster, Michael RP188, RP277  
 Fowler, Alan 77, 78, 79, 80, 82, 84  
 Fox, Lindsay 554  
 Frakes, Robert WP286  
 Franca, Fernanda MP091  
 Francis, Royce 778  
 Francisco, Alex MP179, MP225  
 Franciscon, Elisangela 154  
 Frank, Aaron MP020  
 Frank, Ashlea MP206  
 Frank, Richard 569, WP034, WP044  
 Franklin, James TP057  
 Franks, Diana 466, 467  
 Fraser, Alicia 179  
 Frazier, James 11  
 Freddo, Alessia 762, TP152  
 Frederick, William TP102  
 Fredette, Thomas WP094, MP226  
 Fredricks, Timothy 27, MP201, WP275  
 Freedman, Jonathan 133  
 Freeman, Harold 148  
 Freeman, Jennifer 524  
 Frei, Eva 586  
 Frew, John RP270  
 Fridgen, Christina 45  
 Friedel, Elizabeth 17  
 Friedman, Carey 64, 758  
 Friesen, Kenneth WP004  
 Friske, Peter RP122  
 Fritz, Alyce T. 404  
 Fritz, Ken 193  
 Froggett, Steve 269  
 Frost, Robert 217, TP036  
 Frouin, Heloise 97  
 Fry, Michael 9  
 FU, Jianjie 677  
 Fuchsman, Phyllis 529, WP260  
 Fuentes, David 57, TP028, RP041  
 Fuhrman, Sylvia WP113  
 Fujimine, Yoshinori TP162  
 Fujimoto, Yuji RP096  
 Fujimura, Maho TP184, TP185  
 Fujimura, Moho TP188  
 Fulford, Richard RP261  
 Fulthorpe, R 627  
 Fulton, Michael 394, RP232  
 Funk, David 508, MP041  
 Furlong, Edward 346, 357, TP004  
 Furtula, Vesna MP144  
 Furumai, Hiroaki RP023  
 Gable, Kevin WP109  
 Gaertner, Karin RP264  
 Gagliano, Gregory G. TP165  
 Gagne, Matthew 149  
 Gagnon, Marthe Monique 516, RP014, MP050, WP108, RP258  
 Gago-Ferrero, Pablo 532  
 Galat, David WP115  
 Gale, Robert 513  
 Gallagher, Eugene 693  
 Gallagher, Evan TP059  
 Gallagher, Meaghan RP022  
 Gallagher, Sean 116  
 Gallampos, Christine WP073  
 Gallipeau, Sherrie 418  
 Galvez, Fernando 392.5, WP227  
 Gamal El Din, Mohamed TP114  
 Gamal El-Din, Mohamed 572, WP029, WP043  
 Gamal-El Din, Mohamed 241  
 Gamberg, Mary TP087  
 Gan, Jay RP026, RP027, MP107  
 Gandhi, Nilima WP232, RP244  
 Gangopadhyay, Keshab 736  
 Gangopadhyay, Shubhra 736  
 Gannon, Damon WP110  
 Gannon, John 250  
 Gantner, Nikolaus MP033  
 Gao, Ce 319, TP135  
 Gao, Weimin RP211  
 Gao, Zhi WP191  
 Garber, Kristina 575 MP171, MP208  
 Garcia, Erick 241  
 Garcia, Karla RP225  
 Garcia, Santos RP188, RP277  
 Garcia, Thelma WP025  
 Garcia Gonzalez, Cynthia MP053  
 Garcia-Garcia, Erick 472, TP114  
 Garcia-Reyero, Natalia 29, 224, 377, 633, RP038, RP090, TP112, TP134  
 Gardinali, Piero 395, TP022, TP027  
 Gardiner, William 608  
 Gardner, Bernie RP051  
 Gardner, Murray 285, 286  
 Garmon, Mary RP198  
 Garner, Thomas 623  
 Garret, Robert MP217  
 Garvey, James RP179  
 Gassel, Margy WP025  
 Gathergood, Nick 688, 691, 709, RP055, RP057, RP058, TP219, TP220, TP221  
 Gatian, Nick 396  
 Gauthier, Celina RP134  
 Gauthier, Lewis 167, MP012  
 Gauthier, Thomas TP157  
 Gavin, Qi TP136  
 Gawor, Anya 442, MP004, TP144  
 Gaytan, Brandon WP102  
 Ge, Jun-Qing 472, TP114  
 Ge, Yuan 322  
 Ge, Yue RP078  
 Gebbink, Wouter 167, 257  
 Geel, Alix WP081  
 Geens, Tinne TP143  
 Geerts, Lieve 126  
 Gehen, Sean 332  
 Gendron-Fitzpatrick, Annette WP185  
 Geng, Dawei 259  
 Geng, Xia WP008  
 Gensemer, Robert 125, 275, 276, 662, WP128  
 Gentes, Marie-Line MP009, MP016  
 Genualdi, Susan 62, 475, TP033  
 Genuis, Stepehn 174  
 Gerhards, Reinhard RP047  
 Gerke, Amanda RP264  
 Germano, Joe 397  
 Germida, Jim 470  
 Getzinger, Gordon RP013, MP024, MP111  
 Gevertz, Amanda 647  
 Gewurtz, Sarah 169  
 Ghavre, Mukund TP219  
 Ghosh, Upal 192, 761, MP101, MP174  
 Giacomini, Marina 507  
 Gianessi, Leonard 245  
 Giang, Amanda 556  
 Giardono, Michelle 61, TP233  
 Gibson, Glenys 31  
 Giddings, Jeffrey MP059  
 Gielazyn, Michel 393  
 Giesy, John 27, 105, 471, 568, 719 RP063, WP157, RP181, RP187, MP201, RP254, WP266  
 Giesy, John P 680, TP151  
 Gilbert, Benjamin WP160  
 Gilbert, Beth 555  
 Gilbert, Dorothea WP070  
 Gilbert, Mary 22  
 Gilbert, Samantha 40, RP294  
 Gilio Meina, Esteban MP047  
 Gilliam, Jaime 637  
 Gillis, Martin 573  
 Gillis, Patricia MP040, WP207  
 Gillis, Patty 477  
 Gilron, Guy MP037  
 Gimeno, Sylvia WP125  
 Giordano, Ady RP197  
 Giordano, Rosanna RP109  
 Giroux, Jean MP009  
 Giroux, Jean-Francois MP016  
 Giusti, Arnaud MP150  
 Glaholt, Stephen 635, 636, WP208  
 Glassmeyer, Susan 346, 638, TP004, TP007  
 Gleason, Amber MP030  
 Glenn, Brad 42  
 Glessner, Allison 483, 785  
 Glodzik, Katie 427  
 Glover, Tim RP131  
 Glozier, Nancy 474  
 Gobas, Frank 90, 93, 204, 265, 498, TP049, TP066, TP080, TP081, TP089, TP119  
 Godard-Codding, Celine 562, WP049  
 Godfrey, Adam 31  
 Godtfredsen, Kathy 185  
 Goeritz, Ina TP091  
 Goetz, Frederick 39, 381, TP013  
 Goetz, Rick 379  
 Goff, Kira WP035  
 Gogal, Robert 385, 701, WP112  
 Goh, Kean RP027  
 Gohlke, Julia 629  
 Gold, Arthur 106  
 Gold-Bouchot, Gerardo 258, RP230, MP246  
 Goldberg Day, Amy RP111  
 Golden, Nancy 511  
 Goldsmith, Barbara 424  
 Goldstone, Jared 562  
 Goldstone, Jed 133, 134  
 Gomes, Eduardo TP180  
 Gomez, Luiz MP092  
 Gomez-Alvarez, Agustin MP089  
 Gomez-Eyles, Jose 761  
 Gondikas, Andreas RP292  
 Gong, Changrui TP027  
 Gong, Ping 377, 633, 681, WP263  
 Gonzalez, Liliana 106  
 Gonzalez, Rosalina 387, TP100  
 Gonzalez Rebollar, Sonia MP173  
 Goodfellow, William 109, 110, 281  
 Goodman, Julie MP197  
 Goodrum, Philip MP185, MP227, RP024  
 Goosey, Emma WP180  
 Goovaerts, Pierre MP216  
 Gopalapillai, Yamini WP205  
 Gorbunov, Maxim TP030  
 Gorder, Kyle MP155  
 Gordon, Denise WP137  
 Gordon, Kim 2  
 Gore, Rohitkumar RP057  
 Gorsuch, Joseph 413, RP264  
 Goss, Kai-Uwe 642, 689  
 Gosse, Julie TP115  
 Goto, Yuta TP051, WP245  
 Gottschalk, Fadri 737  
 Gou, Na TP135  
 Gou, Na 621, 626  
 Gou, Na 319  
 Gouguet, Ron 742  
 Gouin, Todd 129, 399, 530, RP029  
 Goulding, Adam 340  
 Gouliarmou, Varvara 89, MP121  
 Gourmelon, Anne 329  
 Grabanski, Carol 91, WP069, TP208  
 Grace, John TP118  
 Grace, Richard TP056  
 Graham, Chris MP082  
 Graham, John MP029  
 Gramatica, Paola 208, 583, 683  
 Grandjean, Philippe 287  
 Grasman, Keith 513, WP113, TP191  
 Grass, David TP027  
 Grassley, James 51, RP018, RP270  
 Grasso, Neal 438  
 Gravenmier, Josh 190, MP179, MP225  
 Gray, Brian WP185  
 Gray, Earl 223  
 Gray, Evan 270  
 Gray, L 122  
 Grear, Jason WP130  
 Greaves, Alana TP052  
 Green, M RP273  
 Green, Mark 783  
 Green, Norman 262  
 Greenberg, Grace MP086, TP234  
 Greenberg, Marc 79, 82, 109, 110, MP237, MP238, MP239, MP240, MP241  
 Greene, Gretchen WP063, RP087  
 Greene, Richard TP046  
 Greenfield, Ben 669, TP216  
 Greenstein, Darrin MP204  
 Greenwood, Spencer RP091  
 Greer, Colleen RP167  
 Greig, Denise RP068  
 Grey, Brian TP053  
 Greytak, Sarah 54  
 Grieger, Khara 733  
 Griffin, Cody MP105  
 Griffith, Joe RP261  
 Griffith, Robert RP295  
 Griggs, Chris 736  
 Grim, Volker 11  
 Grimm, Christiana 70, 550  
 Grimm, Volker 489, 493, 708  
 Grohman, Chelse MP123  
 Grosell, Martin 232, WP131, WP214, WP229  
 Gross, Timothy 752, MP043, RP177, RP212  
 Grover, James WP095  
 Gruber, Steve 278, 279, 743  
 Grue, Christian 51, RP018, RP270  
 Gschwend, Philip 450, 696  
 Gu, April 319, 621, 625, 626, TP135  
 Guan, Bing TP005  
 Guan, YuFeng 260  
 Guarino, Fabio 415  
 Guchardi, John 331  
 Gueguen, Celine WP232  
 Guerrero, Joel MP110  
 Guerrero, Rodolfo RP171  
 Guigueno, Melanie 67  
 Guilhermino, Lucia MP052, TP106



- Guimaraes, Fernando MP076  
 Guimaraes, Laura TP106  
 Guiney, Patrick 335, MP142  
 Gunnarsson, Jonas 760  
 Guo, Jia 535, 718  
 Guo, Rui TP054, TP120  
 Guo, Ying 372  
 Gurbisz, Monika RP058  
 Gust, Kurt 377, 385, 633, TP132, WP254, RP256  
 Gustafsson, Orjan 63  
 Gutierrez, Melida 350  
 Gutjahr-Gobell, Ruth WP130, TP245  
 Guy, Christopher 515  
 Guzman Garcia, Xochitl MP173, MP190  
 Guzman Martinez, Maria del Carmen RP154, RP279  
 Guzman-Garcia, Xochitl MP053  
**H** Ha, Nguyen TP074  
 Ha, Sung Yong 107, TP042, TP047, TP269, RP004, RP034  
 Habel, Mark WP094  
 Habib, Tanwir 377, 633, TP134, TP097, RP038, RP090  
 Habig, Clifford 119, MP205  
 Hable, Whitney 201, MP069  
 Hadad, Christopher RP049  
 Haddad, Tarek RP056  
 Hadjamberdiev, Igor MP199  
 Haffner, Douglas TP090  
 Hafner, Christoph 333  
 Hage, David 159  
 Hageman, Kimberly TP238  
 Hagen, Mariel 241  
 Hagood, Gale 396  
 Hagy, James 661  
 Hahn, Mark 27, 466, 467, 634, 719  
 Hahn, Megan WP174  
 Haiba, Egge TP070  
 Haigh, Ted TP246  
 Hala, David TP211  
 Halden, Norm 227  
 Halden, Rolf 270  
 Halder, Marlies 329, TP085  
 Hale, Beverley WP205  
 Hale, Robert 17, 355, WP187  
 Hale, Sarah 760  
 Haley, Mark TP104  
 Halitschke, Rayko 337  
 Hall, J Christopher WP145  
 Hall, Maurice 593  
 Hall, Maury 695  
 Hall, Tilghman MP206  
 Hall, W. Scott RP140  
 Hallinger, Kris 783  
 Halog, Anthony 3, RP115, RP192, RP284, RP285  
 Halsall, Crispin 65, 68  
 Halstead, Neal 247, 419  
 Hamalainen, Heikki RP213  
 Hamer, Mick WP086  
 Hamers, Robert WP169  
 Hamers, Timo 23, WP075  
 Hamilton, Patrick MP081  
 Hamissou, Mijitaba WP057  
 Hammer, Karen 768  
 Hammer, Katie MP041  
 Hammers-Wirtz, Monika 333  
 Hammerschmidt, Chad TP230  
 Hammock, Bruce RP214  
 Hammond, Larry MP163  
 Hampton, Thomas 465  
 Han, Gi Myung TP042, TP047, TP269, RP034  
 Han, Jin-Su TP199  
 Han, Jun 220  
 Han, Xing TP181  
 Hanamoto, Seiya WP147  
 Hancock, Gregg 724, 725  
 Hancock, Peter RP066  
 Haney, Dennis RP189  
 Hanisch, John 783  
 Hanna, Shannon 141  
 Hanna, Terry WP254  
 Hannah, Robert WP155, TP169  
 Hannigan, Robyn 559, RP052, WP129, WP132, WP134  
 Hansen, Bjørn Henrik 313, 609, 767, RP094, WP140  
 Hansen, James 294, 510, TP173  
 Hansen, Steffen 733  
 Hanson, Mark 227, 588, WP004, TP008, WP144, RP157, TP168, RP238  
 Hanson, Niklas 48, MP198  
 Hanzas, John MP169  
 Hapeman, Cathleen MP096  
 Harbourt, Chris 725  
 Hard, Courtney MP217  
 Hardesty, Douglas MP039, WP224, RP249  
 Harding, Aaron 645  
 Hardy, David 233  
 Hare, Landis WP217, WP218, WP222  
 Haring, Herman 298  
 Harmon, Ashley WP166, RP287, RP291  
 Harmon, Gary TP224  
 Harner, Tom 62, 173, 475, TP120  
 Harney, Jodi TP015  
 Harrad, Stuart MP126, MP127  
 Harrass, Michael RP140  
 Harris, Catherine MP081  
 Hart, Jerry 715  
 Hartig, Phillip 223  
 Hartmann, Andrea TP259  
 Hartnik, Thomas 760  
 Harvey, Ellen 355  
 Harwani, Suhash 455, WP025  
 Harwood, Amanda 88, WP066, MP095  
 Haselman, Jonathan WP055  
 Hashikawa, Ryosuke MP007  
 Hassan, Sayed RP162  
 Hassett, John 61, TP233  
 Hassinger, Christopher 731  
 Hastie, Colin 530  
 Hattum, Bert TP089  
 Haug, Zachary 11  
 Hauri, James TP232  
 Hauschild, Michael 774, WP200, WP233  
 Haver, Darren RP026, RP027  
 Hawari, Jalal 386  
 Hawke, Rebecca RP159  
 Hawkins, Adam WP163  
 Hawthorne, Joseph 321  
 Hawthorne, Steven 91, WP069, TP208  
 Hayase, Daisuke WP211  
 Hayashi, Takehiko RP251  
 Hayashi, Terutake MP007  
 Hayes, Thomas 691  
 Hayes, Tyrone 418, 754  
 Hayhoe, Katharine 187  
 Haynie, Rebecca WP097  
 Hazelton, George RP139  
 Hazelton, Peter RP105  
 Hazlerigg, Charles WP084  
 He, Jianfeng TP040  
 He, Qiang WP175  
 He, Yuhe 568  
 Head, Jessica 378, 379, 381, 560, TP105, TP108, WP184  
 Headley, John 470, 569, 573, WP032  
 Headley, John Vernon WP035  
 Hebert, Armelle RP056  
 Hebert, Craig 167, 257  
 Hecker, Markus 568, RP181, RP187, RP204  
 Hedman, Curtis 436  
 Hedman, Jenny 760, TP077  
 Heger, Sebastian 710, 711, 713  
 Heggelund, Laura 323  
 Heggstad, Ben WP055  
 Heideman, Warren WP169  
 Heiger-Bernays, Wendy 179, MP127  
 Heimbach, Udo 547  
 Heinz, Gary WP181, WP182, WP187  
 Heithmar, Edward 272, TP204  
 Helbing, Caren 220  
 Helfeld, Ruth WP128  
 Helie, Jean-Francois MP016  
 Hellweger, Ferdi WP148  
 Helm, Paul 244, 442, 555, 556, 557, MP008, TP054, MP055, TP144, WP179, WP180  
 Helm, Roger 404  
 Hemmer, Becky 47, MP042, MP048  
 Hemming, Jocelyn RP152, RP293  
 Hemond, Harold 277, 598  
 Hendershot, William 320, 324  
 Henderson, Andrew 777  
 Henderson, Colin 16  
 Henderson, Nicole RP052  
 Hendley, Paul 491, 724, 725, WP091, MP183  
 Henning, Miranda 483, 672, 785, MP130, WP260  
 Henriksen, Thomas 760  
 Henry, Cassandra WP047  
 Henry, Cassie 414  
 Henry, Kevin 724, MP178  
 Henry, Paula WP261, WP270, WP279  
 Henry, Theodore 43, WP098, WP175  
 Henry, Travis 96  
 Henshaw, Alan MP081  
 Hensley, Jerry WP259  
 Hentges, Steven TP001  
 Hentschel, Brian 655  
 Heo, Kakwon TP047  
 Hepner, Brent TP037, MP111  
 Herckes, Pierre 270  
 Hering, Herman TP187  
 Hermanson, Mark 66  
 Hernandez-Calderas, Irma MP053, MP190  
 Hernout, Beatrice 708  
 Herodes, Koit TP070  
 Herr, Natalie 464  
 Herrin, James WP097  
 Herrmann, Jan 273  
 Herve, Jessica 719  
 Herve, Sirpa WP067  
 Heskett, Marvin 58  
 Hesselbach, Renee WP103  
 Heuvel, Michael WP028  
 Hewitt, Mark 569, WP034  
 Hickey, Christopher 404  
 Hickey, Graeme 682  
 Hickmann, Silke 430  
 Hida, Yoshifumi RP096  
 Hidding, Bjorn 129  
 Higashisaka, Kazuma TP184, TP185  
 Higgins, Benjamin 416, 562, WP049  
 Higgins, Christopher 270, 353, 640 TP122, TP201  
 Higgins, Monica RP083  
 Higgins, Sarah MP040  
 Highland, Terry 502, 618, TP071, TP208, WP194, RP100, RP103, RP110, RP266  
 Higley, Kathryn 501  
 Higuchi, Yuta MP233  
 Hill, Elizabeth 449  
 Hill, Jonathan 149, 150  
 Hill, Laura 263  
 Hill, Shaunta 242  
 Hillis, Jeffrey RP179  
 Hillyard, Matthew WP244  
 Hindle, Matthew 219  
 Hintelmann, Holger 36, MP030, MP033  
 Hinton, David RP245  
 Hipolito, Marcio MP091  
 Hirai, Toshiro TP188  
 Hirata, Sawako 705, MP007, WP211  
 Hirose, Akihiko TP270  
 Hitchcock, Kristen MP221  
 Hites, Ronald 311, 536, MP013  
 Hladik, Michelle 243  
 Ho, Kay 145, 446, 452, WP020  
 Ho, NY 333  
 Ho, Shuk-mei 456  
 Hoang, Tham Chung 410, 704, WP286  
 Hoberg, James RP132, WP258  
 Hockett, J 502, RP100, RP103, RP110, WP194, TP208  
 Hockett, James TP071  
 Hockett, Russell 618, RP104, RP266  
 Hodges, Juliet WP014  
 Hodgson, Dave MP081  
 Hodson, Mark RP124  
 Hodson, Peter 169, 195, 196, 197, 198, 199, 200, 448, MP070, WP077, RP167  
 Hoeger, Glenn 389, TP155  
 Hoeninghaus, David MP092  
 Hoff, Dale 502, TP071, WP079, RP100, RP103, WP194  
 Hoffman, Alex 206  
 Hoffman, Joel WP079  
 Hofstetter, Thomas 460  
 Hogan, Natacha 238, 566, 567, WP028, WP033, WP036, WP037  
 Hoguet, Jennifer TP237  
 Hoh, Eunha 655, RP068  
 Hoheisel, Sarah RP103  
 Hoke, Robert 218, TP181  
 Holbrook, R David WP167  
 Holden, Patricia 322  
 Holder, Helen 685  
 Holder, Jennifer MP185  
 Holdway, Douglas 331  
 Holm, Ryan MP123, RP254, WP266  
 Holladay, Jeremy 701, WP112  
 Holladay, Steve 701, WP112  
 Holland Fritsch, Erika 318  
 Hollebone, Bruce WP077, WP190  
 Hollenkamp, Carol RP178  
 Hollert, Henner 333, 710, 711, 713  
 Holm, Patricia 341  
 Holm, Stewart MP043, RP212  
 Holmes, Catherine 123  
 Holmes, Chris WP014, MP183  
 Holmes, James 750  
 Holmes, Jamie 393  
 Holmes, John 285  
 Holmstrup, Martin TP161  
 Holsen, Thomas 164, MP054, MP058, RP064  
 Holt, Pierce MP092  
 Holzer, Brittany TP177, RP195  
 Homering, Margaret 237  
 Hommen, Udo WP083, WP088, RP135  
 Homsher, Michael WP100  
 Hong, Sang Hee 107, RP004, TP042, TP047, TP269  
 Hong, SangHee RP034  
 Hong, Seongjin RP063, TP151  
 Hong, Yongseok 55, TP195  
 Hongwei, Hu 101  
 Honkanen, Jani 606  
 Hook, Tomas 770  
 Hooper, Michael 400  
 Hooser, Emily 249, TP178  
 Hopke, Philip 164, 165, MP058  
 Hopke, Phillip MP054  
 Hoque, Ehsanul TP244  
 Hoque, Md 627  
 Horii, Yuichi RP060, TP148, TP162  
 Horiuchi, Fumie 705  
 Hornbuckle, Keri 370, 539, 541, 551, 552, 756  
 Horneck, Donald RP142  
 Hornung, Michael 22, 26  
 Horowitz, Dorianne Borsay WP130  
 Horrell, Nakita RP020  
 Horsley, Teri RP160  
 Horst, Allison 322  
 Hosaka, Mitsugu TP262  
 Hoskins, Bart 298, WP122  
 Hosmer, Alan WP091, RP157, RP252  
 Hosono, Shigeo 256  
 Hosono, Takahiro TP261  
 Houde, Magali 235  
 Housenger, Justin 575, MP171  
 Houser, Elizabeth 501  
 Hove, Helge 94  
 Hovel, Wendy 189  
 Howard, Cynthia RP134  
 Howard, Philip 171, 208, 641  
 Howell, Nathan 721  
 Hoyett, Zakiya WP242  
 Hsieh, Tsung-Chih RP175

- p>Hsu-Kim, Helen RP245
- 
- p>Hu, Jing TP163
- 
- p>Hu, Xialin MP120
- 
- p>Hu, Xinxin 680
- 
- p>Hu, Yongfeng 573, WP032
- 
- p>Huang, CP WP158
- 
- p>Huang, Da-Ji RP175
- 
- p>Huang, Haiou 349
- 
- p>Huang, Susie MP159
- 
- p>Huang, Zachary WP258
- 
- p>Hubberstey, Andrew MP079
- 
- p>Huber, Gregory RP121
- 
- p>Huddleston, Matt 395, TP022, TP027
- 
- p>Hudelson, Karista RP281
- 
- p>Hudgens, Daniel 427, WP081
- 
- p>Hug, Christine 453, WP073, WP074
- 
- p>Huggett, David 491
- 
- p>Huggett, Duane WP146, WP151, MP170, RP188, TP211, TP271, RP277
- 
- p>Hughes, Kathy 149
- 
- p>Hughes, Lauren TP236
- 
- p>Hughes, Melissa MP029
- 
- p>Hughes, Michael TP138
- 
- p>Hughes, Stacy TP075
- 
- p>Huhtala, Sami TP064
- 
- p>Huijbregts, Mark RP116
- 
- p>Huiyan, Pan TP184
- 
- p>Hull, Matthew WP166
- 
- p>Hull, Peter TP195
- 
- p>Hull, Ruth 576, 782
- 
- p>Hulsey, Thomas 235
- 
- p>Hulstrom, Larry 295, 297
- 
- p>Hung, Hayley TP175
- 
- p>Hung, Silas MP159
- 
- p>Hungerbuhler, Konrad 373
- 
- p>Hunt, Carlton 695
- 
- p>Hunt, John RP107
- 
- p>Huntley, Steven 186, MP185
- 
- p>Huntsman-Mapila, Philippa WP038, WP039, WP040, WP041
- 
- p>Hur, Jeonghyun TP183
- 
- p>Hursky, Olesya RP248
- 
- p>Huset, Carin 367, TP067
- 
- p>Husowitz, Barry RP058.5
- 
- p>Huston, Mark 82
- 
- p>Hutcheson, Michael RP074
- 
- p>Hutchins, Stephen 17
- 
- p>Hwang, Dae-Sik 522
- 
- p>Hwang, Hyun-Min 75, 650, WP003, WP024, TP164, WP178, TP254
- 
- p>Hwang, Sung Hee RP214
- 
- p>Hyeon, Chang 100
- 
- p>Hyobu, Yuika WP211
- 
- p>Hyun, Seunghun RP059
- 
- p>Iancu, Cornel RP054
- 
- p>Iannuzzi, Jacqueline MP177, TP150
- 
- p>Iannuzzi, Timothy 190, TP150
- 
- p>Ibrahim, Lara WP088
- 
- p>Ichihashi, Koichi TP188
- 
- p>Iguchi, Taisen MP078
- 
- p>Ijiri, Shigeo TP213
- 
- p>Ikemoto, Tokutaka 707
- 
- p>Ikenaka, Yoshinori TP213
- 
- p>Ikonomou, Michael 90, 265, TP049, TP056, TP066, TP118, TP119
- 
- p>Ilyas, Muhammad 100
- 
- p>Im, Jong-Kwon MP145
- 
- p>Impellitteri, Christopher 533, TP187
- 
- p>Ingersoll, Chris 82, 228, 229, MP039, MP237, MP238, MP239, MP240, MP241, TP176, WP224, WP248, RP104, RP178, RP249
- 
- p>Innes, Liz 430
- 
- p>Ireland, Scott MP128
- 
- p>Irvine, Cameron MP212
- 
- p>Isaksson, Elisabeth 66
- 
- p>Isham, William 278
- 
- p>Ishizuka, Mayumi TP213
- 
- p>Isobe, Atsuhiko WP220
- 
- p>Isobe, Tomohiko 100, 175, 371, MP007, WP026, TP061, TP074, MP124, TP141
- 
- p>Israelsson, Peter 659, RP048
- 
- p>Itai, Takaaki 371, 705, WP022, TP074, WP211, WP220
- 
- p>Iten, Loren 710
- 
- p>Itoh, Norio TP184, TP185, TP188
- 
- p>Ivanina, Anna WP133
- 
- p>Ivey, Chris RP104
- 
- p>Iwaniuk, Andrew WP282
- 
- p>Iwanowicz, Luke 515
- 
- p>Iwasaki, Yuichi , WP209
- 
- p>Iwata, Hisato 707
- 
- p>Jaber, Mark 116
- 
- p>Jack, Richard 729
- 
- p>Jackman, Paula RP091
- 
- p>Jackson, Allyson 517, WP111
- 
- p>Jackson, Brian 146, TP243
- 
- p>Jackson, Crystal 47, 330, MP048
- 
- p>Jacques, Duane 293, 296
- 
- p>Jager, Tjalling 489
- 
- p>Jahnke, Annika 205, TP218
- 
- p>Jahnke, James TP169
- 
- p>Jakob, Lena 760
- 
- p>Jamting, Asa 273
- 
- p>Jannash, Amber 770
- 
- p>Jannik, Gerald 494
- 
- p>Jansen, Deb WP283
- 
- p>Janssen, Colin 636
- 
- p>Janssen, Elisabeth 759
- 
- p>Jantunen, Liisa 442, 557, TP144, TP175
- 
- p>Janz, David RP205
- 
- p>Janzen, Wolfgang 208
- 
- p>Jardine, Tim TP093
- 
- p>Jarvis, Tayler MP160, WP197
- 
- p>Jasper, Justin 358
- 
- p>Jatar, Muriel RP070, RP071
- 
- p>Jayaraman, Saro TP245
- 
- p>Jeffers, Steven MP218
- 
- p>Jemenez, Begonia TP141
- 
- p>Jenkins, Jeffrey 780
- 
- p>Jenkins, Kenneth MP185
- 
- p>Jenkins, Thomas RP162
- 
- p>Jennings, Cecil 21
- 
- p>Jenny, Matthew 134, 466, 634
- 
- p>Jensen, Allan TP057, MP230
- 
- p>Jensen, Brenda MP112
- 
- p>Jensen, Kathleen 223, 224, 769, WP136, MP138, MP149, RP151, MP151, MP152, RP222
- 
- p>Jensen-Fontaine, Madeleine WP029
- 
- p>Jenson, Correne 502, WP194
- 
- p>Jenssen, Bjorn WP075
- 
- p>Jeong, Seulki WP235
- 
- p>Jeong, Seung-Woo TP183
- 
- p>Jessick, Ashley 137
- 
- p>Jewell, Joe 396
- 
- p>Jho, EunHea TP101
- 
- p>Ji, Hezhe MP188
- 
- p>Ji, Kyunghee TP151, WP157, RP218
- 
- p>Jia, Fang MP107
- 
- p>Jian, Le RP008
- 
- p>Jiang, Guibin 259, RP007
- 
- p>Jiang, Mingshun 697, 699
- 
- p>Jiang, Weiyang RP026, RP027
- 
- p>Jiang, Zhimei MP155
- 
- p>Jin, Yihe 172
- 
- p>Jin, Yongnu TP042
- 
- p>Jo, Hun-Je WP159, WP160
- 
- p>Joachimsmeier, Ina 547
- 
- p>Joaquim-Justo, Celia MP150
- 
- p>Jobling, Susan MP081
- 
- p>Johansen, Oistein TP019
- 
- p>Johns, Mark TP015
- 
- p>Johnson, Boris TP148
- 
- p>Johnson, Bradley 538
- 
- p>Johnson, Brent MP041
- 
- p>Johnson, Brian RP294
- 
- p>Johnson, David 377, 703, TP014, RP081, TP103, RP286
- 
- p>Johnson, Eben 299
- 
- p>Johnson, Heather 234
- 
- p>Johnson, Jane 483, 785, MP130
- 
- p>Johnson, Jean 367
- 
- p>Johnson, Jeffery MP134
- 
- p>Johnson, Jeffrey MP135
- 
- p>Johnson, Josephine 13
- 
- p>Johnson, Kelsey 564
- 
- p>Johnson, Kevin WP149
- 
- p>Johnson, Mark 385, 585, WP254, WP265, WP268
- 
- p>Johnson, Nathan TP095
- 
- p>Johnson, Philip 404
- 
- p>Johnson, Reed 9
- 
- p>Johnson, Rodney WP055, WP079, MP149
- 
- p>Johnson, Sophia TP030
- 
- p>Johnson, Steven 247
- 
- p>Johnsons, Steven 419
- 
- p>Johnston, Craig MP029
- 
- p>Johnston, John MP029
- 
- p>Johnston, Robert 102, MP110
- 
- p>Johnstone, Mary Beth MP180
- 
- p>Jolley, Dianne RP217
- 
- p>Jolliet, Olivier 773, 777
- 
- p>Jonas, Adam MP060
- 
- p>Jones, Alan 162, 308
- 
- p>Jones, Ann 427
- 
- p>Jones, Brenda MP128
- 
- p>Jones, Daniel 96, MP177
- 
- p>Jones, Darryl TP003
- 
- p>Jones, David 570
- 
- p>Jones, Kenneth WP267, WP274
- 
- p>Jones, Kevin 373, TP146
- 
- p>Jones, Kevin C 537
- 
- p>Jones, Paul 568, RP063
- 
- p>Jones, Robert WP276
- 
- p>Jones, Russell 724, 725
- 
- p>Jones, Stephanie 27, 518, 719
- 
- p>Jones, Stephen 362
- 
- p>Jones, Wendelyn 118, 245, 332
- 
- p>Jonker, Michiel WP069
- 
- p>Jordaan, Sarah 771
- 
- p>Jordan, Steve 741
- 
- p>Jorens, Philippe 178, 374, TP143
- 
- p>Jorge, Mariana 507
- 
- p>Joseph, Aley MP146
- 
- p>Jovanovic, Boris 239
- 
- p>Ju, ZhiYong WP060
- 
- p>Julias, Christine 365, TP158
- 
- p>Julshamn, Kaare 94
- 
- p>Jung, Jae-Woong TP101
- 
- p>Jung, Jee-Hyun RP004, RP034
- 
- p>Jung, Jinho RP246
- 
- p>Jung, Se-Young TP199
- 
- p>Jung, Tea-wook 440
- 
- p>Junot, Stephen TP215
- 
- p>Juraske, Ronnie 773
- 
- p>Jurkschat, Kerstin 323
- 
- p>Kabler, Kent MP178
- 
- p>Kaczmar, Swiatoslav MP242
- 
- p>Kagabu, Makoto TP261
- 
- p>Kagota, Keichirou RP144
- 
- p>Kahl, Alandra TP003
- 
- p>Kahl, Michael 223, 224, 769, MP138, MP149, RP151, MP151, MP152, RP222
- 
- p>Kais, Britta 333
- 
- p>Kajenthira, Arani 286
- 
- p>Kajiwarra, Natsuko 175
- 
- p>Kakela, Reijo 59
- 
- p>Kako, Shin'ichiro WP220
- 
- p>Kalkhoff, Stephen 444, TP149
- 
- p>Kalkhoff, Steven 445
- 
- p>Kallarakavumkal Thomas, Jith RP205
- 
- p>Kalnejas, Linda 698
- 
- p>Kaltenecker, Georgina 555
- 
- p>Kalve, Erica MP179
- 
- p>Kamada, Haruhiko TP184, TP185, TP188
- 
- p>Kameda, Yutaka WP147
- 
- p>Kamman, Neil MP029
- 
- p>Kamo, Masashi WP209, RP251
- 
- p>Kamunde, Collins WP215
- 
- p>Kanbara, Chika WP026
- 
- p>Kane Driscoll, Susan 361, 481, 594, 757, MP172
- 
- p>Kang, Seoktae 572
- 
- p>Kang, Seong-Gil MP210
- 
- p>Kang, Young-Min MP145
- 
- p>Kannan, Kurunthachalam 368, 372, WP007, WP008, WP011, TP057, TP061, RP062, MP113, TP140, TP141, TP148, MP154
- 
- p>Kannan, Narayanan TP042
- 
- p>Kapo, Katherine 103, 643, WP015
- 
- p>Karasov, William 236
- 
- p>Karchner, Sibel 27, 466, 634, 719
- 
- p>Karjalainen, Anna TP064, RP213
- 
- p>Karjalainen, Juha TP064
- 
- p>Karlsson, Maja 433
- 
- p>Karnjanapiboonwong, Adcharee TP069
- 
- p>Karouna-Renier, Natalie WP187, WP261, WP270, WP279
- 
- p>Karpman, Matthew 241
- 
- p>Karpowicz, Jessica 475
- 
- p>Karsell, Bill RP120
- 
- p>Kashiwada, Shosaku WP162
- 
- p>Kasuga, Ikuro RP023
- 
- p>Katsiadaki, Ioanna MP166
- 
- p>Katsumata, Masakazu MP181
- 
- p>Katz, Alan WP259
- 
- p>Katz, David 261, WP020
- 
- p>Katzenback, Barbara 241
- 
- p>Kaufmann, Ron WP204
- 
- p>Kaukinen, Karia 240
- 
- p>Kawa, Mary TP240
- 
- p>Kawamoto, Tatsuhiko TP270
- 
- p>Kay, Denise MP180, MP201, RP223, RP254, WP266
- 
- p>Kaye, Sean 655
- 
- p>Kazumura, Kimiko MP181
- 
- p>Keane, Colleen 383
- 
- p>Keating, Jonathan MP031
- 
- p>Keating-Connolly, Janet WP126
- 
- p>Keay, Kenneth 591, 693
- 
- p>Keegan, Michael WP094
- 
- p>Kehrig, Helena RP072, RP073, RP076, MP157
- 
- p>Keil, Karen 8, TP102
- 
- p>Keinanen, Markku TP124
- 
- p>Keir, Micheal 196
- 
- p>Keir, Mike MP056
- 
- p>Keisler, Jeffrey 425
- 
- p>Keiter, Steffen 333
- 
- p>Keith, Richard WP208
- 
- p>Keithmalesatti, Sarun WP188
- 
- p>Keller, Alex 166
- 
- p>Keller, Arturo TP183
- 
- p>Keller, Jennifer TP237
- 
- p>Kellock, Kristen 21
- 
- p>Kelly, Barry 158, 264, 356, TP063, TP212
- 
- p>Kelly, Caryn 686, MP006
- 
- p>Kelly, Valerie 345
- 
- p>Kemble, Nile 228, 229, RP104, MP237, MP238, MP239, MP240, MP241, WP248
- 
- p>Kennedy, Alan 359, 703, 736, TP098, TP103, TP132, WP159, WP166, RP250, RP256, RP287, RP291
- 
- p>Kennedy, Chris TP127, MP144
- 
- p>Kennedy, Christopher 240, 340, TP080
- 
- p>Kennedy, Peter 493
- 
- p>Kennedy, Sean 27, 338, 518, 719
- 
- p>Kennedy, Sharilyn 199
- 
- p>Kennedy, Ted RP170
- 
- p>Kenow, Kevin WP185
- 
- p>Kerby, Jacob 417
- 
- p>Kern, John 485, 514, WP114, MP216
- 
- p>Kern, Matt 118
- 
- p>Kerr, Richard 701, WP112
- 
- p>Keskinen, Tapio TP064
- 
- p>KeskiSaari, Sarita TP124
- 
- p>Kessel, Cristy MP214
- 
- p>Ketelle, Richard RP075
- 
- p>Kettlewell, David 189
- 
- p>Key, Peter 394, RP232
- 
- p>Khan, Bushra RP208
- 
- p>Khan, Eakalak RP080
- 
- p>Khan, Iftheker 732
- 
- p>Khan, Mohammad MP158
- 
- p>Khan, Usman MP143
- 
- p>Khanh, Lam 705
- 
- p>Khim, Jong Seong RP063, TP151, WP157, MP210
- 
- p>Khim, JongSeong 105
- 
- p>Khonsue, Wichase WP051, RP099, WP240
- 
- p>Kidd, Karen 33, 251, 637, MP032, TP081, WP082, TP093, TP194, RP229, RP253
- 
- p>Kierski, Michael 361

- Kiesler, Jeffery 734  
 Kiker, Greg WP078  
 Kim, Aelee RP260  
 Kim, Bo-Mi 139  
 Kim, Chan-Kook TP151  
 Kim, Cheolmin RP260  
 Kim, Hee-Young 458, WP248  
 Kim, Heileen RP292  
 Kim, Hyunseung 75, WP003, WP024, WP178  
 Kim, Hyunyoung WP152  
 Kim, Jaeshin TP231, TP236  
 Kim, Ji-Won WP138, RP246  
 Kim, Jiwon RP260  
 Kim, Jongwoon 678, RP084  
 Kim, Joon-Woo MP124  
 Kim, Jungkon WP138, RP260  
 Kim, Kyungtae 561  
 Kim, Moon-Kyung TP199  
 Kim, Moonkoo RP004, RP034  
 Kim, MyeongSeop RP059, RP169, WP280, WP284  
 Kim, Nam Sook 107  
 Kim, Nam Hyun WP173  
 Kim, Ryoo-Ok 315, 563  
 Kim, Sang Don WP152  
 Kim, Sangdon MP088  
 Kim, Sanghun 678, RP084  
 Kim, Shinwoong 579, WP124  
 Kim, Sun-A WP046  
 Kim, Sunmi WP138, RP260  
 Kim, Tae Hun WP152  
 Kim, Un-Jung 440, 458, WP248  
 Kim, Woo-Keun WP138, RP246  
 Kim, Wookeun RP260  
 Kim, Yang-Hoon TP126, WP141  
 Kim, Yongeun RP059, RP169, WP280, WP284  
 Kim, Younghee 639  
 Kimberly, David WP045  
 Kimbrough, Kimani 168  
 Kimpe, Linda TP247  
 Kimura, Kayoko 239  
 Kimura, Kumiko WP147  
 King, Emily 571  
 King, George WP265, WP268, WP281  
 King, Kerensa RP018  
 King, Morgan WP040  
 King, Susy MP029  
 Kipka, Undine 659, TP031, RP048  
 Kipp, Katrina 298  
 Kipper, Karin TP070  
 Kirby, Margaret TP125  
 Kirchner, Scott TP160  
 Kirchner, Thomas 4  
 Kirichenko, Elmira RP165  
 Kirk, Jane 33, 37, 476, 477, MP030, MP031, MP032  
 Kirkwood, Ashlee RP132  
 Kirman, Chris 722  
 Kish, Nicole RP232  
 Kitana, Jirarach WP051, RP095, WP188, WP240  
 Kitana, Noppadon WP051, RP095, RP099, WP188, WP240  
 Kitao, Kenjiro RP096  
 Klaine, Stephen 42, 376, 623, 738, WP018, TP065, RP081, WP149, WP153, WP161, RP286  
 Klaper, Rebecca 39, 147, 436, MP148, WP176  
 Klasky, John WP123  
 Klecka, Gary 117, TP001  
 Kleeman, Patrick WP054  
 Klein, Heather 549  
 Klein, Ronald 495  
 Klerks, Paul RP173, RP247, RP255  
 Kliegman, Sarah 653  
 Klosterhaus, Susan 166, RP068, MP192  
 Knafla, Anthony RP121, TP154  
 Kobayashi, Yuko MP181  
 Koch, Iris 702, RP127, WP193, WP196  
 Koelliker, Mathias 632  
 Koh, Chul-Hwan MP210  
 Kohli, Mohan WP034  
 Koike, Takashi MP181  
 Kojima, Naoya MP228  
 Kolb, Ruth 279  
 Kolb Ayre, Kim 104  
 Kolic, Terry 555, MP008, MP055  
 Kolok, Alan 24, 137, RP013  
 Kolpin, Dana 346, 357, TP004  
 Komatsu, Shohei MP191  
 Komori, Hiroaki TP141  
 Komori, Kenjiro 705  
 Konkel, Greg MP206  
 Kool, Pauline 323  
 Koopman, Heather WP110  
 Koporec, Kevin 81  
 Korampally, Venumadhav 736  
 Korber, Darren 44  
 Kornacki, Alan 217, TP035  
 Korte, Joseph WP055  
 Kosarac, Ivana TP229  
 Kosian, Patricia WP055  
 Kost, Elias 737  
 Kostamo, Auli WP067  
 Kostich, Mitch 452, 638  
 Kostich, Mitchell 254, 301  
 Kosugi, Yuki 256, TP262, TP270  
 Kountzman, James WP236  
 Kovarich, Simona 683  
 Kozerski, Gary WP009  
 Kracunas, Alison TP265, WP273  
 Krahforst, Christian 596  
 Kramer, Vincent 117, MP064, MP065, MP163  
 Kraus, Johanna 504  
 Krauss, Martin 453, WP073, WP074  
 Kreinberg, Allison TP267  
 Kreis Jr, Russell 488  
 Kreitinger, Joseph 359  
 Kriehuber, Ralf 334  
 Kroeger, Keith MP202  
 Kroger, Eric MP135  
 Kroger, Robert 542, MP105, RP185  
 Kroll, Kevin 20, 314, 317, 342, 382, RP015, TP123, WP174  
 Kronberg, Leif RP044, TP062  
 Krueger, Henry 116  
 Krueger, Jaane WP021  
 Krupka, Betty 365  
 Krupka, Elizabeth TP160  
 Krystek, Petra MP017  
 Ku, Wen 659  
 Kubiak, Tim 515  
 Kubitz, Jody 395, TP022, MP123  
 Kubwabo, Cariton TP229  
 Kucklick, John RP068, WP110, TP237  
 Kuemmerer, Klaus RP056  
 Kuhn, Anne RP257  
 Kuhn-Hines, Anne RP239  
 Kuhne, Wendy 494  
 Kuivila, Kathryn 243, 246, WP054, TP176  
 Kukkonen, Jussi 326, 606, 660, TP064, MP100, TP124  
 Kulacki, Konrad 142  
 Kullman, Seth MP137  
 Kumagai, Michio WP211  
 Kumar, Niraj 580, 628  
 Kümmerer, Klaus 586  
 Kummrow, Fabio WP076, RP201  
 Kung, Tiffany TP111, TP113  
 Kunisue, Tatsuya TP061, MP113, TP140, TP141  
 Kunito, Takashi 707  
 Kunz, James RP104, TP176, RP178, WP224, MP237, MP238, MP239, MP240, MP241  
 Kuo, Dave TP084  
 Kuo, Jen-ni RP036  
 Kuperman, Roman 386, TP099  
 Kurata, Takahiro RP096  
 Kuribayashi, Shuta RP096  
 Kurzanski, Paul 781  
 Kutil, Nicholas 50  
 Kwak, Inn-Sil MP194, MP211  
 Kwak, Jin-Il 579  
 Kwak, Kyunghee WP157  
 Kwon, Jin-Wook MP103, MP115  
 Kwon, Seokjoon 761  
 Kylin, Henrik 255, 442, 443, RP043, TP144  
 La Belle, Bruce WP025  
 La Guardia, Mark 355  
 Lachenauer, Erica TP128  
 Lagadic, Laurent MP150  
 Lage, Fernanda WP199  
 LaGorga, John RP024  
 Lahren, Tylor TP071  
 Lahti, Marja TP062  
 Lai, Hao-Feng 265, TP049  
 Lai, Steven RP066  
 Laird, Jennifer RP081, TP132, WP159, WP163, WP166, RP250, RP256, RP286, RP291  
 Lake, James RP239, RP257  
 LaLone, Carlie MP149, RP222  
 Lambert, Jason MP236  
 Lambert, Matthew 553, 755  
 Lambert, Veronique MP087  
 Lamichhane, Kiran RP161  
 Lamoree, Margaretha 23, RP056, WP075  
 Lamothe, Paul 504  
 Lamoureux, Beth 189  
 Lamoureux, Elizabeth TP083  
 Lamparelli, Marta WP243  
 Lampe, Karen RP139  
 Lampi, Mark 335  
 Lan, Jiaqi 621  
 Lance, Stacey WP267, WP274  
 Landers, Stephen RP266  
 Landis, Wayne 104, 108, 403, WP090, RP101, RP269  
 Landrum, Peter 607, WP065, MP095, MP131  
 Lane, Douglas TP120  
 Langley, Sean WP039  
 Langlois, Maureen 545  
 Langseth, David 438, TP096  
 Lanno, Roman RP049, RP146, WP238, WP276  
 Lapczynski, Aurelia 528, WP016  
 LaPoint, Thomas RP161  
 Lara-Flores, Maurilio TP255  
 Larive, Cynthia 461  
 Larose, Catherine TP044  
 Larsen, Henrik WP233  
 Larsen, Mark 189  
 Larsen, Martin 652  
 Larsson, Ake 48  
 Lasier, Peter RP105, RP162  
 Latimer, Henry 637  
 Lattier, David WP137  
 Lau, Christopher TP053  
 Laudon, Hjalmar 68  
 Lauer, Mariana 408, WP202, WP203  
 Lavado, Ramon 461, TP059  
 Lavelle, Candice WP174  
 Lavoie, Daniel MP134, MP135  
 Lavoie, Emma 581, TP240  
 Lavoie, Michel WP231  
 Lavoie, Raphael MP015  
 Law, Sheryl MP084  
 Lawrence, John 44, 434, WP035  
 Lawson, Greg MP030, MP032  
 Lazarus, Rebecca WP187  
 Lazorchak, James 17, 29, 193, 254, 298, 301, 531, 533, 558, 638, MP041, WP137, TP170, TP171, TP187, TP260  
 Le, Chris WP029  
 Le, Thai-Hoang TP126, WP141  
 Le Faucheur, Severine RP031  
 Lead, Jamie WP001  
 Leather, Jim MP110  
 Lebeuf, Michel 195, 196, 197  
 LeBlanc, Garrett RP226  
 LeBlanc, Heidi 665  
 Leder, Christoph RP056  
 Lee, Bill 171, MP057  
 Lee, Boknam MP137  
 Lee, Byeong-Gweon WP173  
 Lee, Carol RP138, RP141, RP220  
 Lee, Cindy 98, 307, WP010, WP027, MP104  
 Lee, Dong Soo WP046  
 Lee, Dong-Jin MP088  
 Lee, Eunkyoung 300  
 Lee, Henry WP281  
 Lee, Holly 252  
 Lee, Jae-Seong 136, 139, 315, 469, 520, 522, 561, 563  
 Lee, Ji-Woo TP009  
 Lee, Jin-Wook RP246  
 Lee, Jinwook RP260  
 Lee, Kathy 769, MP138  
 Lee, Kyu Tae TP151  
 Lee, Kyu-Seung MP103, MP115  
 Lee, Linda 18  
 Lee, Michael MP165  
 Lee, Myun Joo WP152  
 Lee, Sang-Woo RP246  
 Lee, Sanghyup WP159, WP160  
 Lee, Sangwoo WP138, TP151, RP260  
 Lee, Sum Chi 173  
 Lee, Sun-Hong MP088  
 Lee, Sung Kyu RP246  
 Lee, Sung-Eun RP176  
 Lee, Sung-Kyu TP126, WP138, WP141  
 Lee, Sungha WP173  
 Lee, Sungkyu RP260  
 Lee, Wen-Yee 157, TP002, TP258  
 Lee, Woo-Mi 579, WP124, WP171  
 Lee, Woojin TP151  
 Lee, Young-Mi 136, 469, 563  
 Lee, Yun-sik RP059, RP169, WP280, WP284  
 Lee, Yunah WP046  
 Lee-Steere, Christopher 9  
 Leet, Jessica 18  
 Leeuwen, Stefan TP057, TP089  
 Leffler, John 763  
 Legare, Benoit 200, MP075  
 Leggett, Michael 118, 245  
 Lehman, Christine 117  
 Lehmann, Daniel Wade WP118  
 Lehnerr, Igor 37  
 Lehr, Randy 727  
 Leifer, Anne WP128  
 Leigh, Katrina WP260  
 Leigh, Mary Beth 610  
 Leinonen, Greta 326  
 Leirvik, Frode 216, TP019  
 Leisle, Dwight MP110  
 Lemes, Marcos MP158  
 Lemmetyinen, Juha TP124  
 Lenihan, Hunter 141  
 Leonard, Erin 506  
 Leonards, Pim 19, 23, 93, MP017, WP075, TP089  
 Leonel, Juliana TP043  
 Leopold, Annegaaik 116  
 Leppanen, Matti 59, 326, 606, TP064, WP067, MP100, TP124, RP213, TP218  
 Leprince, Pierre MP150  
 Lepsic, Robert 479  
 Lesage, Pascal RP119  
 Lescord, Gretchen 251, MP032  
 Leslie, Heather 93, TP089  
 Lesmeister, Sarah RP174  
 Lesperance, Mary 220  
 Lester, Deborah 729  
 Letcher, Robert 167, 169, 257, 338, 565, MP009, MP012, MP014, MP015, MP016, TP052, WP272  
 Letourneau, Lisa WP198  
 Leung, Maxwell 133  
 Leung Liu, Lucie TP116  
 Lev, Steven RP022, MP023, RP125, RP129, WP237  
 Levac, Joshua 67  
 Levi-Polyachenko, Nicole 40, RP294  
 Levin, Leonard 284  
 Levin, Penny WP060  
 Levine, Ed 391  
 Levine, Steven 123  
 Lew, Mindy MP146  
 Lewis, Ari WP239  
 Lewis, Michael MP093



Lewis, Phillip 13	Loftus, Matthew 467	MacLeod, Matthew 205, 312, 373	Martinez-Jeronimo, Felipe Fernando TP179, RP190
Lewis, Solange 524	Loganathan, Bommanna TP039	MacManus-Spencer, Laura TP235, TP265, WP273	Martinovic-Weigelt, Dalma 221, RP011, TP109, TP110, RP151
Li, Aimin TP010, TP011, TP012	Loguinov, Alex RP038, WP102	MacNair, Doug MP083	Martins, Camila 507
Li, Belinda TP118	Lohman, Kristen 284	Macolly, Elizabeth 79, 84	Martins Lopes, Thais TP045
Li, Fasong WP008	Lohmann, Rainer 69, 255, 654, 755, 758	MacPherson, Karen MP008, MP055	Martinson, John 462, 638
Li, Guiying WP189	Loibner, Andreas 89	MacRae, Russ WP120	Marty, Sue 123
Li, Hongxia 244	Lonabaugh, Kevin WP251	Macwan, Vanessa TP160	Martyniuk, Christopher 219, 342, 727, MP072, TP127, TP129, TP130
Li, Huizhen MP099	Long, Kevin 187	Maddalena, Randy 76, 312	Maruya, Keith RP068
Li, Li 699, 700	Loomis, Mark MP128, TP206	Maddox, Catherine WP261, WP270	Marvin, Chris MP008, TP054
Li, Loretta TP118	Loper, John 80, 84	Maenpaa, Kimmo 59, TP064, WP067, TP218	Maryoung, Lindley 400, TP059
Li, Miling RP235	Lopes, Melissa RP237	Magali, Rodrigues , RP010, MP187, MP213	Mash, Heath TP004, TP006, TP007
Li, Nanqin MP108	Lopez, Gabriela RP263	Magaw, Renae 543	Mason, Rob 35
Li, Shibin TP186	Lopez, Jacqueline 635	Magee, Brian 2, 127, 364, 389, 390, 480, WP126, TP155, MP220	Masset, Mathew TP102
Li, Xiangdong 604	Lopez-Romero, Faviel TP179	Magome, Shinya WP220	Masson, Greg 751
Li, Xiaolin 657	Lopp, Donna MP087	Magruder, Chris 436	Mast, M 441
Li, Yingming 259	Lorentz, Warren 82	Maher, Edward 499	Masterin, Brian 278, 279, 743
Liang, Daan TP023	Lorenzen, Kai WP084	Mahler, Barbara 366, 439, TP156	Mastrocco, Frank 130
Liangmin, Huang RP077	Loro, Vania MP040, MP049	Mair, Rachel RP249	Mastrota, Nicholas 575, MP171, MP208
Liao, Chunyang WP007	LoSchiavo, Andrew 425	Maity, Suman 770	Masunaga, Shigeki 256
Libby, Alan RP239	Lotufo, Guilherme 152, 384, MP239	Majewski, Michael 439, 444, 445, TP149	Mather, Tamsin 285
Libby, P 590	Louis, Kacie WP169	Major, Andrew 515	Matherne, Brian 32, MP092
Libby, Scott 697	Loureiro, Susana 323	Major, Kaley RP109	Mathew, Rooni 503
Liber, Karsten 471, RP186	Love, Natalie 125	Makino, Masakazu TP257	Mathews, Teresa RP075
Lichtveld, Maureen 779	Lowe, Edgar 751	Makynen, Elizabeth 224, 769, MP138, MP149, MP151, RP151, MP152, RP222	Mathis, Michael MP142, MP162
Lie, Elisabeth WP075	Lowe, John 291, 292, TP173	Malarvannan, Govindan MP124	Matlock, Daniela MP161
Liess, Matthias 401	Lozano, Nuria 303	Malekani, Kalumbu TP167	Matlock, Marty RP085
Lillegard, Kathryn WP055	Lozano, Sylvain MP001	Maletz, Sibylle 711	Matousek, John MP123, RP254
Lillenberg, Merike TP070	Lu, Connie 500	Mallet, Claude TP242	Matson, Cole 46, WP161, TP202
Lillicrap, Adam 329, MP170	Lu, Jian 726, TP068	Mallory, Mark 31, 263	Matsumoto, Jacque MP140
Lim, Atalie , TP117	Lu, Linghong 220	Malm, Olaf RP072, RP073, RP076, MP157	Mattman, Andre TP121
Lim, Dhong-il 107	Lu, Xiaoqiao RP142	Malone, Don 479	Mauck, Robert WP110
Lim, Dongyoung RP290	Lu, Xiufen WP029	Mancini, Cecilia WP192	Maul, Jonathan RP200, RP206, RP210, RP211
Lim, Eun-Suk RP246	Lu, Yonglong 105, RP063	Mancein, Rachata RP099	Maung, Emily TP252
Lim, Eunsuk RP260	Luchmann, Karim 132	Manheimer, Kelly MP133	Maus, Christian 15
Lim, Puy TP063	Ludwig, David 77, 78, 82	Manik, Yosef 3, RP284	May, Thomas 513, RP050
Lim, Suk-Kyung MP103	Luck, Jenna 443	Mann, Reinier 415	Mayer, Foster WP118
Lin, Bin-Le WP087	Lugo-Ibarra, Karina TP264	Manna, Joseph RP139	Mayer, Philipp 89, 205, WP067, WP070, MP121, TP161, TP218
Lin, Pinpin MP159	Luis, Luis MP052	Mannetje, Andrea MP126	Mayfield, David MP207, WP239
Lin, Xin Xin TP128	Luke, Nai-Chia 365, TP158	Manning, Andrew 405	Mayo, Michael 377
Lin, Yan 368, WP008	Lukey, Natasha WP061	Manning, Gillian 27, 518	Mazurkiewicz, Paul 685
Lin, Youjian 726	Luna, Luis RP201	Mannings, Mark 31, 263	Mazzei, Meagan RP129
Lin, Zhifen RP265	Luna, Tamara MP141	Mano, Hiroyuki 138	Mazzotta, Marisa 745
Lindberg, Ty 274, RP245	Lundebye Haldorsen, Anne-Katrine 94	Mao, Dazhi WP014	McArdle, Margaret 481, MP172
Lindsay, James 703	Luo, Xiaosan 604	Maranduba, Henrique RP113	McArdle, Meg MP222
Lindstrom, Andrew TP053	Luo, Yang WP099	Marano, Grazia 586	McAvoy, Drew 640
Linford, Katherine RP022, RP128	Luthy, Richard 85, 759	Marantonia, Adriana MP091	McBride, Murray MP087
Lingenfelter, Susan 515	Lutz, Charles 188	Marchuk, Jascha TP109, TP110	McBride, Toby TP164
Link, Jane 514, WP114, WP253	Luxton, Todd 628	Mareci, Tom 343	McCabe, John RP062
Linkov, Igor 1, 5, 422, 423, 425, 428, 429, 500, 733, 734, 786, WP078, RP086, RP089, WP094 ,	Lydy, Michael 88, WP065, WP066, MP095, MP131, TP177, RP179, RP200, RP221, TP259	Marek, Rachel 370	McCarthy, Christopher 292, 294, TP173
Linton, Tyler WP118	Lynch, Jeniffer 275	Marfil-Vega, Ruth 22, 254	McCarthy, Kathleen 345
Lintott, Darlene RP121	Lyndall, Jennifer 529	Margie, Peden-Adams 235	McCarthy, Kathy 344
Lipton, Joshua 393, WP119	Lyon, Bonnie 348	Margni, Manuele RP116	McCarthy, Melissa 40, RP208, RP294
Liscio, Camilla 449	Ma, Hongbo 41	Marino, Damian RP032, TP133	McCarthy, Sharon 297
Lister, Andrea MP045, MP047, MP073	Ma, Jane MP084, MP214, MP222	Marino, Troy 117, MP163	McCarty, Harry TP225
Little, Brandon WP276	Ma, Qingli 645	Marins, Luis MP077	McClean, Colin 708
Little, Edward 421, MP038, TP176, WP223, WP226, WP230, RP272	Ma, Wai MP098	Mark Welch, David 466, 634	McClean, Michael 179, MP127
Liu, Charlene 365, TP158	Ma, Yuning MP013	Markkarian, Ralph 395, TP022	McClellan-Green, Patricia MP051, RP234
Liu, Fengjie 312	Maage, Amund 94, 262	Markiewicz, April 104	McConkey, Brendan WP044
Liu, Jingfu 268, MP005, MP011	Mabe Jr., Donald TP037	Markin, Melanie 291, 292	McConnell, Laura L. MP096
Liu, Liang-Ying 260	Mabury, Scott 252, 253, MP021, TP054, TP057, RP067	Markus, Meringer 453	McCoole, Matthew 222
Liu, Rui 268	Macaulay, Laura RP013, MP111	Markwiese, James 296	McCormick, Stephen 523
Liu, Sijin RP007	MacDonald, Don MP238, MP240	Marlatt, Vicki TP127, TP129	McCoy, Dan 279
Liu, Tsung-Yun MP159	MacDonald, Donald 82, 228, 229, MP241	Marquez Bravo, Lydia MP087	McCreary, Ricardo 157
Liu, Wei 172, RP007	MacDonald, Gillian 238, WP028, WP037	Marra, Peter 70, 550	McCrinkle, Robert MP012
Liu, Xiao TP212	MacDonald, Gord RP137	Marsden, Lauren RP294	McCulloch, Wayne 281
Liu, Xiaoshan RP218	MacDonald, Ian 112	Marshall, Stuart 433	McDonald, Dave 298
Liu, Ya WP008	MacDonald, Robie 97	Marston, Lauren 40	McDonald, Thomas WP139
Liu, Ying TP072	MacFarlane, John 696	Martellini, Tania 537	McEachern, Preston 473
Liu, Yu-Ting RP245	MacGillivray, Ronald RP028	Martin, Benjamin 489	McElroy, Anne 237, 694
Livingstone, Kelly WP206	Machado, Anderson Abel TP045, TP180	Martin, Erik 56	McEvoy, John RP080
Lizotte, Richard MP223	Macias-Zamora, Jose Vinicio TP264	Martin, Jonathan 173, 241, 255, 471, 472, 568, 571, WP031, WP043, TP114, TP121	McFadden, Lisa 117
Lloyd, Alun 679	Maciolek, Nancy 591, 693	Martin, Lawrence 744	McFarlan, Kelly 608
Lloyd, Shannon 778	Mackay, Chris MP006	Martin, Lynn 247, 419	McFarland, Craig 385
Lo, Bonnie TP127	Mackay, Donald 204, 309, WP019, TP231, TP236	Martin, Pamela 167, MP015	McFarlin, Kelly 610
Lo, Justin TP080	Macke, Dana WP164	Martin, Phillip WP100	McGeer, James 143, WP206, WP228
Lobscheid, Agnes 776	Mackintosh, Cheryl 189	Martin, William 698	McGeer, Jim 412
Locey, Betty 390, 783, TP155	MacLatchy, Deborah MP045, MP046, MP047, MP073	Martinez, Andres 552, 756	McGillis, Wade 535
Locke, Martin RP088		Martinez, Karell 717	McGrath, Joy 395, TP022
Lockwood, Richard RP140		Martinez, Pablo MP077	
Loerks, Julia 713		Martinez Gomez, Diana RP171	

- McIlwain, Brenda 435  
 McInerney, Kevin 160  
 McInnes, Mark 656  
 McInnis, Rodney 477  
 McIntosh, Lisa 297  
 McKay, Kyle RP089  
 McKee, Mike WP115  
 McKenzie, Mary WP063  
 McKnight, Diane 405  
 McKone, Thomas 312, 402, 776  
 McLachlan, Michael 205, TP218  
 McLamore, Eric WP165  
 McLaughlin, Sean TP167  
 McMahon, Paul 597  
 McMahon, Taegan 247, 419  
 McManus, Paddy WP145  
 McMaster, Mark 380, 474, 569, TP130, RP209, TP250  
 McMeechan, Melissa RP069  
 McMennamy, George RP163  
 McMurry, Scott 248, 249, TP178  
 McNeill, Kristopher 653  
 McNeill, Laurie 548  
 McNeill, Sean 566  
 McNett, Debra MP035  
 McPhedran, Kerry TP263  
 McQueen, Andrew 395, TP022  
 McShane, Heather 320, 324  
 McWayne, Megan WP054  
 Meadows, Inga MP218  
 Meadows, John RP050  
 Mearns, Alan 391  
 Medlock, Elizabeth MP149  
 Meeks, Barbara TP132, WP159, WP166, RP256, RP287  
 Meeks, Dawn RP250, WP271  
 Mehinto, Alvina 221, 314, 382, TP123  
 Mehler, W RP221  
 Meier, John 193  
 Meierjohann, Axel RP044  
 Meisel, Leonor TP166  
 Mekenyan, Ovanes 208  
 Melby, Nick RP250  
 Meldrum, Blair 701  
 Melymuk, Lisa 555, 556, 557, WP179  
 Mendenhall, Scout 548  
 Mendes, Mykel 728, TP094  
 Menn, Fu-Minn WP175  
 Menzie, Charlie 192, 361, 403, 481, MP167, MP172, MP222, WP252  
 Merad, Myriam 426  
 Merrifield, Daniel 43  
 Merten, Amy 427  
 Mertens, Birgit 126  
 Messing, Paul RP202  
 Metcalfe, C 45  
 Metcalfe, Chris 244, 435, 627, TP223, TP244  
 Metcalfe, Tracy 435, TP223  
 Metzger, Bernhard 611, MP020  
 Meyer, Carolyn MP085  
 Meyer, Joel 133, TP125, MP203, RP292  
 Meyer, Joseph 77, 78, 413, MP085  
 Meyer, Michael MP137, WP185  
 Meyers, Alison RP136  
 Meylan, Bill 208  
 Micallef, Fabian 76  
 Michael, Holly 351  
 Michel, Jacqueline 391  
 Michelson, Kyle 387, TP100  
 Middleton, Matthew WP249  
 Mierau, Kerry RP149  
 Mierzejewski, Jessica RP189  
 Mierzykowski, Steven 515, WP255  
 Miglino, Andrew TP100  
 Mihaich, Ellen 123, 332, RP193  
 Mihele, Cristian 475, 476  
 Milani, Danielle 672  
 Miles, Mark 9, MP062, MP064, MP065  
 Miles, Scott 391  
 Miller, Aroha TP077  
 Miller, David 91, 380, 488, WP069, TP208  
 Miller, Douglas TP252  
 Miller, Eric MP029  
 Miller, Jason 316  
 Miller, Jeffrey 221  
 Miller, Jonte 692  
 Miller, Julie RP047  
 Miller, Kristi 240  
 Miller, Lesley WP249  
 Miller, Marcus MP019, RP196  
 Miller, Pamela 383  
 Miller, Robert 141  
 Miller, Thomas MP217  
 Miller Neilan, Rachael WP093  
 Millero, Frank WP131  
 Milligan, Michael 164, 165, MP054, MP058  
 Mills, Ken WP082  
 Mills, Lesley TP245  
 Mills, Marc 22, 193, 194, 254, 298, 301  
 Millward, Geoff 509  
 Mils, Rebecca 348  
 Min, Jiho TP126, WP141  
 Minarik, Thomas RP011  
 Minella, Lauren RP160  
 Minet, Emmanuel TP137  
 Mingoa, Robert TP181  
 Minomo, Kotaro RP060  
 Minton, Lindsey WP057  
 Minyard, Morgan 386, TP099  
 Miranda Arce, Maria MP190  
 Misato, Kazuki TP188  
 Miskewitz, Robert 535  
 Miszczak, Ewa RP126  
 Mitchell, Scott 241  
 Mitchell, Shannon RP046  
 Mitrano, Denise TP201  
 Mittal, Kritika 560  
 Mittelman, Anjulice 735  
 Miyake, Yuichi TP162  
 Mizukawa, Hazuki MP007, WP026  
 Mo, Hyoung-ho RP169, RP176, WP280  
 Mocan, Lucian RP054, RP288  
 Mocan, Teodora RP054, RP288  
 Mochizuki, Kousuke MP233  
 Moe, Jannicke 401  
 Moeckel, Claudia 373  
 Moeller, Axel TP040, TP041  
 Moeller, Benjamin 464  
 Mohler, Rachel 543  
 Moldovan, Remus RP288  
 Molinare, Alexis 783  
 Moller, Axel MP018  
 Mompelat, Sophie RP056  
 Mompalaisir, Georges-Marie TP203  
 Monk, Mark MP023  
 Monosson, Emily MP153  
 Monroe, William RP207  
 Monteiro, Carlos MP028  
 Montero, JuanIgnacio RP283  
 Montie, Eric 343  
 Moody, Mary 577  
 Moon, Hee Sun WP235  
 Moon, Hyo-Bang 458  
 Moore, Alyssa WP113  
 Moore, Barry 165  
 Moore, Dana 615 RP102  
 Moore, David 743  
 Moore, Dwayne MP169, MP175  
 Moore, Margo TP080  
 Moore, Matthew RP088, MP223  
 Moore, Michael 593, 695  
 Moore, Richard MP029  
 Moors, Amanda TP237  
 Morales, Daniel WP076  
 Moreira, Isabel RP073, RP076  
 Mori, Takahide TP188  
 Moriarty, Thomas 9  
 Morishita, Yuki TP184, TP185  
 Morita, Junpei WP147  
 Moriwake, Aaron WP060  
 Morris, Adam TP087  
 Morris, Jeff 299, 393, WP119  
 Morris, Kevin MP189  
 Morris, Stephen 630  
 Morrison, Ann Michelle 361, 481  
 Morrison, Hawa WP106  
 Morrissey, Christy 67, 748, RP012  
 Morse, Dave MP055  
 Mortensen, Spencer WP257  
 Mortimer, Juliet RP029  
 Moseley, Arthur 218  
 Mosher, Paul WP060  
 Motegi, Mamoru 256  
 Mount, Andrew 738  
 Mount, David 502, 618, TP071, RP097, RP100, RP103, RP104, RP110, TP208, RP266  
 Mourad, Faraj 234  
 Moustafa, Ahmed 568, 572  
 Mroz, Rita 191  
 Mu, Dongyan 714  
 Mu, Yunsong 677  
 Mucha, Amy 193, 194  
 Mueller, Amy 277  
 Mueller, Claudia TP087  
 Mugunthan, Pradeep 659  
 Muir, Derek 33, 66, 67, 169, 171, 235, 251, 255, 399, 476, 641, MP008, MP030, MP031, MP032, MP056, MP057, TP081, TP087, MP117  
 Mukwede, Evelyn MP155  
 Muller, Erik 141, TP132  
 Muller, Kirsten WP044  
 Muller Hoff, Mariana TP180  
 Mundorf, Christopher 779  
 Munkittrick, Kelly 380, 637 MP045, MP047, MP072, RP098, TP130, TP194, MP209, RP259  
 Munley, Kathleen WP131  
 Munney, Kenneth 515  
 Munns, Wayne 487, 661, 741  
 Murata, Sayaka TP061  
 Muresan, Adriana RP288  
 Murfitt, Roger 491  
 Murimboh, John RP130  
 Muroya, Taro TP213  
 Murphy, Cheryl 378, 379, 381, TP013  
 Murphy, Gregory 95, MP109  
 Murphy, Ian MP232  
 Murphy, Stephen WP061  
 Murray, Deborah 181  
 Murray, Jacolin RP068  
 Murray, Karen 213  
 Musante, Craig 321  
 Muscalu, Alina MP055  
 Muth-Koehne, Elke 334  
 Muto, Mamoru WP022  
 Muttray, Annette 316  
 Muzandu, Kaampwe TP213  
 Mwaanga, Phenny 140  
 Nabb, Diane TP181  
 Nabeshi, Hiromi TP184, TP185, TP188  
 Nacci, Diane 54, 135, 462, 466, 630, MP029, RP239  
 Nadeau, Michael MP123, MP201  
 Nadella, Sunita WP219  
 Nadin, Ulrich 453, WP072  
 Nadon, Tanya MP047  
 Nagano, Kazuya TP184, TP185, TP188  
 Nagasaka, Seiji WP162  
 Naile, Jonathan 105, TP151, P063  
 Nair, Prakash 325, WP142, WP143, RP290  
 Nakada, Norihide WP147  
 Nakae, Dai TP262  
 Nakagame, Yuya WP162  
 Nakajima, Fumiyuki RP023  
 Nakamura, Jun TP163  
 Nakamura, Mitsuhide RP096  
 Nakashima, Etsuko WP220  
 Nakata, Haruhiko TP051, TP061, TP141, TP261, WP245, RP184  
 Nakayama, Shoji 22, 254, 298, 301  
 Nakayama, Shouta TP213  
 Nakazawa, Koyomi MP228, MP235  
 Nalepa, Thomas 770  
 Nam, Dong-Ha 336, 339, 378, 560, TP105, TP108, WP184, TP196  
 Nam, Kyoungphile TP101, WP235  
 Nam, Sun-Hwa WP172, RP203  
 Narumiya, Masanori WP147  
 Nascarella, Marc TP097  
 Nascimento, Iracema 712, RP113  
 Nasu, Masao TP184, TP185  
 Natale, Guillermo TP133  
 Nava Montes, Alma RP154, RP279  
 Navarrete, Jessica RP225  
 Neal, Andrea WP025  
 Neal, Barbara 123, 332, MP163  
 Nearing, Michelle RP127  
 Nedoff, Judy MP179  
 Neels, Hugo 176, 178, 374, MP126, TP142, TP143  
 Neely, Robert 427, WP081  
 Neff, Jerry 605, 607  
 Neheli, Tannis 316  
 Nei, Lembit TP070  
 Neidig, Marie 554  
 Neilson, Andrew WP177  
 Nelson, Jessica 367  
 Nelson, Robert 212, 696  
 Nelson, William 161  
 Nestler, Eric 594  
 Neto, Jose RP113  
 Neupane, Binod RP115  
 New, Julie RP022, WP237  
 Newman, Mark 658  
 Newsted, John MP201, RP223, WP253, RP254, WP266  
 Newton, Jason RP012  
 Newton, Kim Newton WP161  
 Newton, Sarah 763  
 Ng, Carla 399  
 Ng, Raymond 220  
 Nguyen, Hang 182  
 Nguyen, Khai RP163  
 Ni, Wanmin WP099  
 Nice, Helen RP014  
 Nicell, Jim MP143  
 Nicholls, Ian MP003  
 Nichols, John 206, TP085, TP210  
 Nicks, Diane RP199  
 Nicol, Elizabeth MP081  
 Nie, Xiangping WP189  
 Nietch, Christopher 533, MP041  
 Nikolakis, Alexander 10  
 Nilsen, Elena RP228  
 Nilsen, Frances MP112  
 Nipper, Marion 696  
 Nisanian, Mandana WP112  
 Nisbet, Roger 141, 489, TP132, RP256  
 Nishidome, Asuka RP184  
 Nishimura, Tetsuji WP150, TP262, TP270  
 Nishioka, Marcia WP013  
 Niwa, Takuto WP162  
 Nixon, Zach 427, WP081  
 Niyogi, Soumya WP221  
 Nizzetto, Luca 534  
 Njoyim Tamungang, Estella Buleng RP233  
 Noel, Lynn WP109  
 Noguchi, Takako 705  
 Nogueira, Lygia MP040, WP207  
 Nogueira, Marta MP028  
 Nogueira, Raquel RP201  
 Nojiri, Kiyoshi 256, RP060  
 Nomiya, Kei 457, MP007, WP026, TP074  
 Norberg-King, Teresa 335, 618, TP071, RP100, RP103, RP110, TP208, RP266  
 Nordtug, Trond 609, 767, RP094, WP140  
 Noriega, Mary 346  
 Norman, Steve RP087  
 Norris, Charles WP100  
 Northam, Wes WP251  
 Norwood, Warren 477  
 Nose, Nasato TP141  
 Nota, Benjamin 631  
 Notch, Emily MP071  
 Nowack, Bernd 737  
 Nowlin, Weston RP273  
 Noyes, Pamela 400  
 Nudi, Adriana TP048  
 Nutter, Brian TP023

Nyberg, Elizabeth MP218  
 Nybom, Inna MP100  
 Nyman, Marianne 544  
**O** O'Boyle, Randy MP214  
 O'Bryan, Ervin TP170, TP171  
 O'Connell, Kimberly 730  
 O'Connell, Steven TP246  
 O'Connor, George 352, 640  
 O'Connor, Megan TP235  
 O'Driscoll, Nelson 31, 33, MP026, MP027, MP032, RP130  
 O'Reilly, Kirk TP159  
 Oakes, Ken TP251  
 Oakes, Robyn 61, TP233  
 Oaks, Lindsay 753  
 Oates, Peter 659, RP048  
 Oberg, Tomas , MP003  
 Ocab, Charlene RP153  
 Occhialini, James MP193  
 Ochiai, Mari WP026  
 Odendaal, James WP005, WP006  
 Odenkirchen, Edward 575, MP171, MP208  
 Oei, Marcella 655  
 Oen, Amy 86  
 Ogata, Akio TP262  
 Ogino, Yukiko MP078  
 Ogle, R Scott 614  
 Ogura, Toshinobu TP184, TP185  
 Oh, Jeong Eun 458, WP248  
 Oh, Jung-Eun 440, TP009  
 Oh, Sung Hee 458  
 Oh, Sung-Hee TP009  
 Oh, Sunghee 440  
 OHara, Eileen WP060  
 Ohlendorf, Harry 670, 749  
 Ohman, Klas RP039  
 Ohtsuka, Nobutoshi RP060  
 Ohura, Takeshi TP148  
 Oikari, Aimo TP062  
 Oka, Tomohiro WP150  
 Okonski, Alexander 149  
 Okoro, Hussein WP005, WP006, RP243  
 Oladiran, Ayoola 241, 472, TP114  
 Olanrewaju, Olujimi WP006  
 Oldi, John WP011  
 Oleksiak, Marjorie 463, 466  
 Oliaei, Fardin 720  
 Olivares, Alba 532  
 Oliveira, Camila WP203  
 Oliveira, Ken 201, MP069  
 Oliveira, Patricia TP106  
 Oliveira, Rafael 151  
 Olmstead, Allen 18, WP055, WP079  
 Olsen, Anders 609, 767, RP094, WP140  
 Olsen, Anthony TP225, TP226  
 Olsen, Marian TP239  
 Olson, James WP049  
 Olson, Terese RP083  
 Olsvik, Pal WP140  
 Olszyk, David WP281  
 Olujimi, Olanrewaju WP005  
 Onnis-Hayden, Annalisa 319, TP135  
 Ono, Masahiko TP261  
 Onorato, Dave WP283  
 OOkura, Hideya RP096  
 Oorts, Koen RP227  
 Opeolu, Beatrice WP005, WP006  
 Orazio, Carl 70, 513, 550, RP050  
 O'Reilly, Kirk 543  
 Oris, James WP136, RP156  
 Orishikida, Takanori TP261  
 Orlando, James 243  
 Ormerod, Steve RP012  
 Ornostay, Anna TP127  
 Orrego, Rodrigo 331  
 Orrick, Greg 242  
 Ortego, Lisa 120, 123, 332, MP161  
 Ortiz, Anna TP002  
 Ortuno-Arzate, Teresa RP268  
 Osachoff, Heather MP144  
 Osborne, Erin MP139  
 Osborne, Juliet 493  
 Oseagulu, Nicole 718

Osemwengie, Lantis 531  
 Ostaszyk, Kim 430  
 Ostaszewski, Arthur RP062  
 Ostrom, Peggy TP075  
 Othman, Mohd Sham WP240  
 Otsuka, Masanari WP022  
 Otte, Jens C 333  
 Otter, Ryan MP082, RP191, RP207  
 Ottermanns, Richard 333  
 Ottinger, MaryAnn 519, WP186, WP256  
 Otton, Susan TP080  
 Ouellet, Jacob WP221  
 Overjordan, Ida 609  
 Overmyer, Jay 10, MP178  
 Overrein, Ingrid WP140  
 Overturf, Carmen WP146, WP151  
 Overturf, Matthew WP146  
 Oviatt, Candace 106  
 Ownby, David RP025, RP125, RP129, WP237  
 Owojori, Olugbenga WP262  
 Owsianiak, Mikolaj 774, WP200, WP233  
**P** Pace, Heather TP201  
 Pacepavicius, Grazina 196  
 Paden, Norka 125  
 Padilla, Stephanie 375  
 Padilla Torres, Juan RP268, RP279  
 Paganini, Christianne TP045  
 Pagano, James 164, 165, MP054, MP058  
 Page, David 607  
 Painter, Roger MP106  
 Paiva, Teresa MP091  
 Pakarinen, Kukka 326  
 Pala, Franco 699, 700, TP017  
 Palace, Vince 227, RP253  
 Palic, Dusan 239  
 Palmer, Rachel TP115  
 Palmer, Susan 116  
 Palmqvist, Annemette WP085, WP086  
 Palumbo-Roe, Barbara RP124  
 Pan, Huiyan TP185  
 Pancorbo, Oscar RP074  
 Pangallo, Kristin 460  
 Panger, Melissa 30, MP176  
 Panko, Julie MP221  
 Pannu, Ravinder MP026  
 Papa, Ester 208, 583, 683  
 Papastavros, Efthimia 159  
 Pape, Jenny TP056  
 Papoulis, Diana 382, WP115, TP123, RP199  
 Paquet, Louise 386  
 Paquet, Nathalie WP213  
 Paquin, Paul 228, 229, 503, RP182, MP229  
 Parekh, Mansi 343  
 Pargee, Suzanne 125, RP108  
 Park, Bradley RP253  
 Park, Jeongim 639, TP151  
 Park, Jerryang 714  
 Park, Jinsoon TP151  
 Park, June Woo WP098, WP175  
 Park, June-Soo 455, 456, WP025  
 Park, Kiyun MP194, MP211  
 Park, Sun Young WP142, WP143  
 Park, SunYoung 325, RP290  
 Parke, Neil TP169  
 Parker, David TP072  
 Parker, Wayne MP140, TP251  
 Parkin-Kullman, Jane 601  
 Parks, Ashley 145, 271  
 Parks, Brad WP080  
 Parra, Amanda TP258  
 Parrott, Joanne 474, 477, WP030  
 Parthasarathy, Peethambaram 457  
 Passamani, Fernanda WP050, TP214  
 Pastorok, Robert WP080  
 Patel, Ami TP107  
 Patel, Parina MP017  
 Patenaude-Monette, Martin MP009  
 Paterson, Mike WP082  
 Patino, Reynaldo RP228  
 Patnaude, Michael WP258  
 Patnode, Kathleen 515  
 Pattanayek, Mala MP224

Patten, Kim WP064  
 Patterson, Heather TP026  
 Patureau, Dominique TP073  
 Paul, John 661  
 Paul, Katie 22  
 Paul, Piuly MP174  
 Paule, Armelle 44  
 Pauclick, Margot WP273  
 Pauwels, Stan WP122  
 Pawlack, Marcin RP071  
 Pawlak, Marcin WP038, RP070  
 Pawlisz, Andrew RP061, TP153  
 Pawlowski, Sascha MP162  
 Payton, Paxton TP182  
 Peart, Tom MP057  
 Peddinghaus, Sabine 333  
 Pedersen, Joel WP169  
 Pedersen, Signe WP086  
 Pedersen, Sindre 768  
 Peers, Jennifer WP119  
 Pell, Judith 493  
 Pellerin, Brian 38  
 Pellerin, Jocelyne 200  
 Pelletier, Derek 785, MP130  
 Pelletier, Emilien RP006, RP009  
 Pelletier, Eric 338  
 Pelletier, Jonathan TP115  
 Pelletier, Marguerite 106, 145, 452, WP020  
 Pelz, Oliver TP027  
 Pembroke, Ann 594  
 Pena, Angelina TP166  
 Penfold, Larry 58  
 Penn, Tony WP081  
 Pennell, Kurt 735  
 Peranginangin, Natalia MP178  
 Percival, H 752  
 Pereira, Gloria RP012  
 Pereira, Solange 712  
 Perendeci, Nuriye TP073  
 Perez, Angela MP146  
 Perez, Leonidas 471, 568  
 Perez-Estrada, Leonidas 472, TP114  
 Pergantis, Spiros 272  
 Perine, Jeff MP178  
 Perkins, Christopher 512, 728, TP025, TP094  
 Perkins, Daniel MP178  
 Perkins, Dawn RP293  
 Perkins, Edward 29, 224, 377, 385, 633, TP014, TP097, TP112, TP132, TP134, WP254, WP263, RP038, RP090, RP256,  
 Perkins, Robert 608, 610  
 Perlanger, Judith 534  
 Perovich, Laura 74, WP013  
 Perron, Monique 145, 261, 446, 452, WP020, TP227  
 Perruchon, Elyse WP260  
 Peru, Kerry 470  
 Pessah, Isaac 318  
 Petering, Dave WP103  
 Petering, Louise WP103  
 Peterman, Paul 70, 513, 550, RP050  
 Peters, Andrew 656  
 Peters, Ruud 266  
 Petersen, Elijah 326  
 Peterson, Mark RP075  
 Peterson, Richard WP169  
 Peterson, Samuel 524  
 Peterson, Stephanie RP266  
 Peterson, Steven MP189  
 Petreas, Myrto 455, 456, WP025  
 Petrich, Nicholas 539, TP147  
 Petroni, Ricardo 659  
 Pettis, Jeffery 13  
 Peyton, David RP278  
 Pfaller, Stacy TP004  
 Pfeiffer, Danielle TP169  
 Pfeeger, Thomas WP281  
 Pflugfelder, Jochen 11  
 Pfrender, Michael 635  
 Phalen, Laura 238, 567, WP028  
 Pham, Diem 182, 299  
 Philbeck, Ray RP166

Phillips, Bryn 447, 764, RP107  
 Phillips, Carlton TP104  
 Phillips, John RP223  
 Phillips, Patrick 357  
 Phillips, Vanessa 255  
 Phopase, Jaywant TP220  
 Picard, Christian TP167  
 Pierce, Katy 234  
 Pierce, Samuel MP105  
 Pieroni, Michelle TP043  
 Pierron, Fabien 202, 468  
 Pietari, Jaana 757, TP159  
 Pierrick, Michael RP248  
 Pina, Benjami 532  
 Pinheiro, Natalie 483  
 Pinkerton, Kent 400  
 Pinkney, Alfred 515, RP025  
 Pirela, Herbert MP189  
 Pirooznia, Mehdi 633  
 Pistorius, Jens 10, 547  
 Plachn, Bill 549  
 Plautz, Stephanie RP168  
 Plocher, Milton WP265, WP268, WP281  
 Plouffe, Genevieve 706, 775  
 Poda, Aimee 736, TP098, WP159, RP287  
 Podhasky, Paula RP138, RP141, RP220  
 Poe, Michael WP194  
 Poezevara, Guillaume MP001  
 Pohll, Greg 645  
 Poirier, David , RP102, TP207  
 Poirier, Michel 191  
 Poissant, Laurier MP027  
 Pontasch, Mandee 413  
 Ponton, Dominic WP222  
 Poole, Gary 476  
 Poot-Cruz, Gilberth TP255  
 Porcher, Celine WP213  
 Porterfield, David WP165  
 Portier, Ralph J 360  
 Portis, Lisa 145, 452, WP020  
 Porvari, Petri TP077  
 Posthuma, Leo 667  
 Postma, Erik 632  
 Poteat, Monica 505  
 Potter, Dave MP012  
 Pourrezaci, Parastoo WP029, RP042  
 Powell, Alisha TP197  
 Powell, David 99, MP035, TP081, RP182, RP194, TP231, TP236  
 Powell, Michael 343  
 Powers, Kevin WP174  
 Poynton, Helen 559, WP129, TP187  
 Prado, Valentina RP118  
 Prats, Eva TP112  
 Preau, James MP119  
 Prego, Ricardo TP253  
 Presley, Steve 187  
 Presley, Steven RP211  
 Preuss, Thomas 489, WP088  
 Preziosi, Damian WP080  
 Price, Bradford WP019  
 Price, Cynthia WP249  
 Price, David TP124  
 Price, Oliver WP014, RP029  
 Price, Richard 359  
 Priestster, John 322  
 PrietoConaway, Maria TP078, TP079  
 Proestou, Dina 462, 630, WP020  
 Prosser, Ryan WP144  
 Provatas, Anthony TP025  
 Prowse, Terry MP033  
 Pruden, Amy 131  
 Prudente, Maricar MP124  
 Pruell, Richard TP245  
 Pryke, Doug MP222  
 Pugh, Rebecca TP237  
 Puglis, Holly 421, MP038, WP223, WP226, RP272  
 Pulster, Erin 525  
 Purdy, Irene 744  
 Puricelli, Sara 92  
 Purrucker, Tom 575, MP171  
 Putt, Russell 114



- Puttaswamy, Naveen WP038  
 Puttaswamy, Naveen WP039, WP040, WP041  
 Pyle, David 285  
 Pyles, Sharlyne MP178  
 Pyper, ML TP073  
**Q** Qi, Xiubin 57, TP028, RP041  
 Qian, Shuang-Yi 653  
 Qin, Guangqiu RP211  
 Qiu, Jingfan TP011, TP012, MP116  
 Qu, Shen RP274  
 Quast, Lauren MP156  
 Quinlan, Erin 533  
 Quinn, Michael 385, WP254  
**R** Ra, Kongtae 561  
 Raach, Meriem 197  
 Rader, Kevin 230, 597  
 Radloff, Kathleen 351  
 Raffel, Thomas 247, 419  
 Ragheb, Magdi 6  
 Rahaman, Obaidur MP002  
 Rail, Jean-Francois MP015  
 Raimondo, Sandy 47, MP042, MP048  
 Raimundo, Joana TP253  
 Raine, Jason WP030, RP204  
 Rainwater, Thomas TP230  
 Raldua, Demetrio TP112  
 Ralph, Allison 341  
 Ralston-Hooper, Kimberly 218, RP013  
 Ramage, Robert TP136, TP139  
 Ramirez, Mark 303, MP096  
 Ramirez, Noemi MP246  
 Ramirez Romero, Patricia MP053, RP154, MP173, MP190, RP268, RP279  
 Ramirez Trejo, Veronica MP053  
 Ramirez-Alvarez, Nancy TP264  
 Ramirez-Islas, Martha RP268  
 Ramirez-Miss, Noemi RP230  
 Ramirez-Vargas, Brenda 258, WP246, TP255  
 Ramos, Claire 512, TP172, WP277  
 Ramos, Kenneth 363  
 Ramsdell, Howard RP172, WP285  
 Ranasinghe, Ananda MP204  
 Rand, Amy 253  
 Rand, David 135  
 Rand, Gary 395, 410, 704, TP022, TP027, WP286  
 Ranville, James 270, 413, TP201  
 Rao, Apparao 623  
 Rao, P Suresh 714  
 Rashleigh, Brenda 488  
 Rasmussen, Joe TP093  
 Rastegar, S 333  
 Raterink, Lisa MP135  
 Rattner, Barnett WP187  
 Rauschenberger, Richard 751, 752  
 Rawlings, Jane 329  
 Rawson, Christopher 516, RP014, WP108  
 Raya-Rodriguez, Maria MP187, MP213  
 Raya-Rodriguez, Maria RP010  
 Rayburn, James TP032, WP057  
 Raza, Ghulam WP001  
 Rea, Anne 739, 741  
 Reboucas, Marcio 712  
 Redden, Anna 31  
 Reddy, Christopher 113, 212, 460, 696  
 Reddy, Tirumuru 17, WP137  
 Redman, Aaron 395 TP022, RP182, MP229  
 Reed, Melissa 490, MP195  
 Reese, Robert MP105  
 Reese, William 95, WP192  
 Regan, Christine 477  
 Reible, Danny 756  
 Reid, Brian 762, TP152  
 Reid, Jennifer 470, WP042  
 Reid, Kim 690  
 Reif, John 235  
 Reifferscheid, Georg 333  
 Reilly, Patricia TP038, WP109  
 Reilly, Timothy 243  
 Reimer, Kenneth 702, RP127, WP193, WP196  
 Reinecke, Adriaan MP196  
 Reinecke, Sophie MP196  
 Reiner, Eric 555, MP008, TP054, MP055  
 Reiner, Jessica TP075  
 Reiner, Reiner TP120  
 Reist, Jim MP033  
 Reitzel, Adam 134, 466  
 Reller, Sandra MP104  
 Remington, Richard 514, WP114  
 Rencz, Andy RP122  
 Replinger, Suzanne 185  
 Retamal, Mauricio RP197  
 Reub, Greg RP087  
 Revelas, Eugene 397  
 ReVello, Rhiannon TP149  
 Revill, Andy 57, TP028, RP041  
 Rex, Andrea 589  
 Reynolds, John 525  
 Reynolds Reid, Kim WP239  
 Rezende, Carlos RP072  
 Rhee, Alexandra 455, 456  
 Rhee, Jae-Sung 136, 139, 561, 563  
 Rhodes, Jeanne MP217  
 Rhomberg, Lorenz MP197  
 Rhyne, Andrew WP134  
 Riady, Adi 100  
 Riar, Navneet MP044  
 Riazanov, Alexandre 219  
 Ribaud, Lisa MP087  
 Rice, Charles 235, 392.5  
 Rice, Clifford 303  
 Rice, Kenneth 752  
 Rich, Courtney 353  
 Richards, Sean TP008, TP168, RP238  
 Richardson, Jason TP111  
 Richardson, Joseph TP039  
 Richardson, Norman MP020  
 Richardson, Stephen TP163  
 Richman, Lisa 672  
 Richter, Catherine 382, TP123  
 Richter, Pablo RP197  
 Ricker, Rob 393  
 Rickwood, Carrie WP038, WP039, WP040, WP041, RP070, RP071  
 Riebach, Paul TP256  
 Riege, Laura 397  
 Rifai, Hanadi 721  
 Rifkin, Erik 55, TP195  
 Rigaud, Cyril 200, MP075  
 Rigg, David 190  
 Rimoldi, John TP268  
 Ringwood, Amy 40, RP208, RP294  
 Rinner, Brian 630  
 Riscassi, Ami 38  
 Rispoli, Fred RP082  
 Ritter, Christina RP145  
 Ritter, Kerry MP204  
 Ritter, Roland 373  
 Rivera, Ignacio WP204  
 Rivera, Josep 717  
 Rivera, Michelle RP237  
 Rivera-Austrui, Joan 717  
 Rivera-Duarte, Ignacio RP155  
 Roark, Shaun TP206, RP254, WP266  
 Robb, Joe MP036  
 Robbat, Albert 646, RP001  
 Roberts, Aaron 32, 738, MP092, WP168, RP281  
 Roberts, Emily 144  
 Robertson, Cory 685  
 Robinson, Eleanor 163, MP122  
 Robinson, Janet 295  
 Robinson, Keith MP029  
 Robinson, Shona RP067  
 Robinson, William 559, WP129  
 Robson, Matthew 555, 556, 557, WP179  
 Rocha, Beatriz TP258  
 Roche, Pascal RP056  
 Rocheleau, Sylvie 386  
 Rochman, Chelsea 655  
 Rodenburg, Lisa 535, 718  
 Rodenburg, Zachary 170, 539, 552  
 Rodgers, John RP145, RP147, RP164  
 Rodney, Sara 484, MP169  
 Rodolakis, Tony WP127, WP241  
 Rodrigues, Aurelie TP106  
 Rodriguez, Ignacio RP015  
 Rodriguez, Lia TP217  
 Rodriguez, Paola TP217  
 Rodriguez Fuentes, Gabriela WP135, RP280  
 Rodriguez Gil, Jose Luis WP144  
 Roe, Amy 201, MP069  
 Roe, Susan 484  
 Roelke, Daniel WP095  
 Roelofs, Dick 631  
 Rogers, Emily WP098  
 Rogers, Jim WP096, RP276  
 Rogers, Richard MP068  
 Rognerud, Sigurd TP077  
 Roh, JiYeon RP078  
 Rohr, Jason 247, 404, 419  
 Roig, Benoit RP056  
 Roland, Victor MP102  
 Roling, Jonathan RP275  
 Roloson, Scott WP033  
 Romano, Tracy 235  
 Romanowski, Lian WP030  
 Romansic, John 247  
 Ronchin, Rebecca RP217  
 Ronco, Alicia RP032, TP133  
 Rorvik, Siv 609  
 Rosabal, Maikel WP217, WP218  
 Rosal, Charlita TP203  
 Rose, Jane RP074  
 Rose, Kenneth WP093  
 Rose, Robyn 10, 13  
 Rosen, Gunther 384, 730, RP155, WP204  
 Rosenbaum, Ralph 774, WP233  
 Rosenblum, Laura TP006, TP007  
 Rosenburg, Bruno 255  
 Rosi-Marshall, Emma RP170  
 Rosman, Lisa 485  
 Rosnack, Kenneth RP066, TP242, TP266  
 Ross, Andrew 57, TP028, RP041  
 Ross, David TP138  
 Ross, Derek MP189  
 Ross, John MP192  
 Ross, Matthew 571, WP031  
 Ross, Peter 97, 240, 340  
 Ross, Tim 116  
 Rossman, Sam TP075  
 Rossmann, Ronald WP185  
 Rouleau, Claude RP006, RP009  
 Rouse Campbell, Kym WP062, WP063  
 Rowan, David 497  
 Rowe, Mark 534  
 Rowland, Steven 570, WP031  
 Roy, Julie 44, 434  
 Roy, Kelly RP075  
 Roy, Maitreyee 273  
 Roy, Nirmal 52, 467  
 Roy, Partha 683  
 Roy, Pierre-Olivier RP116  
 Royer, Laurel TP020  
 Royer, Scott 436  
 Ruan, Xiaodan WP148  
 Ruberti, Nicole WP121, RP131  
 Rucki, Agnieszka WP201  
 Rudd, Murray 430  
 Rudel, Ruthann 74, 304, WP013  
 Rudis, Deborah 612  
 Rudisill, Catherine MP217  
 Rumbold, Darren WP283  
 Runnalls, Tamsin MP081  
 Rus, David 305  
 Rushing, Todd 703  
 Russell, Amber TP097  
 Russom, Christine 617  
 Rust, Michael RP026, RP027  
 Rutkiewicz, Jennifer 560, TP108, WP184  
 Rutter, Allison MP132  
 Rutter, Hannah 47, MP048  
 Ruus, Anders 262  
 Ryan, Adam 228, 229, 232, 413, WP159, RP181, RP216  
 Ryan, Caitlin RP242  
 Ryan, Charlie 2  
 Ryan, Christopher 573, WP032  
 Ryan, Jim 129, WP154  
 Ryde, Ian MP203  
 Ryder, Mark TP090  
 Rygielwicz, Paul WP265, WP268  
 Rytli, Randall 293, 296  
 Ryu, Jongseong TP151, MP210  
**S** Saba, Tarek 649  
 Sable, Shayne WP093  
 Sabo-Attwood, Tara 20, 732, RP013  
 Sacker, Dominic WP155  
 Sadowski, Jathan WP105  
 Sager, Shawn 479, 784  
 Saili, Katherine TP107  
 Sainio, Pirjo TP064  
 Sajwan, Kenneth WP023, TP039, TP146  
 Sakach, Elizabeth 357  
 Salamova, Amina 536, MP013  
 Salatas, Johanna MP222  
 Saleem, Ammar TP247  
 Saleh, Navid 732, RP082  
 Salice, Christopher WP045, WP048, WP058, WP059, TP060, WP093, MP141, RP168  
 Salierno, James RP237  
 Salley, Dara 61  
 Salvadori, Daisy 155  
 Salvito, Daniel 203, 528, WP016  
 Samanda, Ella MP119  
 Samel, Alan 613  
 Samotus, Olivia WP041  
 Sample, Bradley 291, 292, 294, WP093, TP173  
 Samson, Rejean RP119  
 Samuelsson, Goran 760  
 Sanchez, Sarah WP148  
 Sanchez-Hernandez, Juan 415  
 Sanchez-Marin, Paula 409  
 Sandau, Court 56  
 Sanderson, Hans 180, 375  
 Sandra, Pat RP066  
 Sandy, Andy 535  
 Sangster, Jodi 24, WP177  
 Santore, Robert 228, 229, 413, 503, 603, RP181, MP229  
 Santos, Ignes RP076  
 Santos, Jennifer WP050, TP214  
 Saponaro, Sabrina 92  
 Saraceno, JohnFranco 38  
 Sato, Yukiko MP181  
 Saulo, Jordi 717  
 Savin, Tania TP217  
 Savoy, Lucas 512, 517  
 Sawyer, Jennifer RP244  
 Saxe, Jennifer WP017  
 Sayers, Lee MP147, MP164, MP165  
 Sayler, Gary WP175  
 Scanlan, Leona TP128, WP160  
 Scanlon, Kelly 778  
 Scarlett, Alan 570  
 Schaanning, Morten 86  
 Schaefer, Adam 235  
 Schaefer, Karl 473  
 Schaefer, Christoph 334, TP091  
 Schaeffer, Andreas 713  
 Schafer, Michael 83, TP046  
 Schaidler, Laurel 304  
 Schantz, Michele TP237  
 Schauer, James RP293  
 Schaumann, Gabriele 678, RP084  
 Scheef, Gregor MP139  
 Schell, John 80  
 Schenck, Kathleen TP004, TP006, TP007  
 Schenke, Detlef 547, WP021  
 Scherer, Gerhard TP137  
 Scheringer, Martin 373  
 Scherr, Heidi MP200  
 Scherschel, Craig 217, TP036  
 Schiefer, Gregory 109, 110  
 Schierz, Petra Ariette 145, 271  
 Schiff, Kenneth 676, MP204  
 Schiller, Viktoria 334  
 Schimel, Joshua 322  
 Schirmer, Kristin 375

## AUTHOR INDEX

Schlaepfer, Martin TP200  
 Schlechtriem, Christian TP088, TP091  
 Schlekat, Tamar 96, 479  
 Schlenk, Daniel 461, MP044, TP059, RP214, RP274  
 Schmerfeld, John 517, WP111  
 Schmidt, Stine WP070, TP161, TP218  
 Schmidt, Travis 504, 663, 668  
 Schmieder, Patricia WP079  
 Schmitt, Christopher WP107, WP116, WP117  
 Schmitt, Walter 492  
 Schneider, Anne 711  
 Schneider, Suzanne 116  
 Schnorbach, Hans 15  
 Schnute, William MP019, RP196  
 Schoch, Nina TP191  
 Schock, Tracey 225, 763  
 Schoenfuss, Heiko 221, 357, RP011  
 Schofield, Judith MP216  
 Scholz, Nathaniel RP021  
 Scholz, Stefan 375  
 Schoyen, Merete 262  
 Schrag, Daniel 771  
 Schreck, Carl WP119  
 Schuck, Meghan MP202  
 Schulmeier, Jennifer 32  
 Schultz, Melissa 357, RP011  
 Schultz, Sandra WP187  
 Schultz, Terry 28  
 Schulz, Timothy 551  
 Schulze, Tobias 453, WP073, WP074  
 Schumitz, Denise 160  
 Schur, Andrea 14  
 Schuurmann, Gerrit 208  
 Schwab, Kellogg 349  
 Schwartz, Matt RP199  
 Schwierzke-Wade, Leslie Schwierzke-Wade 525  
 Schwindt, Adam 431  
 Scofield, Arthur TP048  
 Scott, Brian 169  
 Scott, David WP267, WP274  
 Scott, Heidi TP223  
 Scott, Paul MP221  
 Scott-Dupree, Cynthia 11  
 Scroggins, Rick 577, 620, MP061  
 Scully, Pamela 79, 81, 82  
 Seager, Thomas 714, WP105, RP118  
 Sebastien, Robert 10  
 Secord, Anne 515  
 Seda, Brandon 738  
 Sedlak, David 358  
 Seegar, William TP025  
 Seeley, Paul 291  
 Segner, Helmut TP085  
 Seibein, Kerry RP295  
 Seidl, Sara MP149  
 Seiler, Thomas-Benjamin 711, 713  
 Seiter, Jennifer 703, WP276  
 Seixas, Tercia RP072, RP073, RP076, MP157  
 Seki, Yuri TP257  
 Selck, Henriette 203  
 Selden, Sara MP227  
 Selin, Noelle 64, 282  
 Selinger, Evan WP105  
 Sellers, Kathleen 731  
 Seo, Jihyun TP151  
 Sepulvado, Jennifer TP122  
 Sepulveda, Maria 770, WP165  
 Sepulveda, Marisol 18, 524, TP187  
 Serbst, Jonathan RP239, RP257  
 Sereby, Joanna WP145  
 Servos, Mark WP034, MP047, TP130, RP209, TP250, TP251  
 Seston, Rita 99, MP035, TP081, RP194, MP201  
 Seth, Rajesh TP263  
 Setiawan, Iwan 100  
 Sett, Amy MP057  
 Sette, Carla TP048  
 Sevdol, Florence 81  
 Seymour, Lynne TP209  
 Shabnam, Rabiya RP080  
 Shafer, Martin RP293  
 Shaffer, Amy MP082, RP191  
 Shah, Vishal 580, 628, RP082  
 Shams, Leyla 509  
 Shankie, Erin 189  
 Shanley, James 38  
 Shanley, Jamie MP029  
 Shappell, Nancy 17, 302  
 Sharma, Manu 526, 644, 648, 690  
 Sharpley, Andrew RP185  
 Shaw, Dimple TP242  
 Shaw, Joseph 465, 635, 636, MP071, WP208  
 Shaw, Patrick 97  
 Shayler, Hannah MP087  
 She, Jianwen 369, TP136, TP139  
 Shea, Damian 213  
 Sheets, Larry 120  
 Shelley, Lesley 240, 340  
 Shen, Li MP008, MP055  
 Shepherd, Andrew WP100  
 Sherrard, Rick MP177  
 Sherrard, Ryan TP129  
 Sherry, James 316  
 Shibata, Tomoaki RP096  
 Shichi, Ryoko MP191  
 Shiflett, Alicia WP254  
 Shikata, Norito TP061, RP184  
 Shim, Won Joon 107, RP004, TP042, TP047, TP151, TP269  
 Shim, WonJoon RP034  
 Shimada, Jun TP261  
 Shimasaki, Seiya WP245  
 Shin, Hyunsook WP138  
 Shin, Key-il RP169  
 Shin, Kyung-Hoon 561, 563  
 Shin, Yu-Jin WP170  
 Shinohara, Ryu-ichi TP061, RP184  
 Shinohara, Ryuichi TP141  
 Shivel, Micheal 84  
 Shivel, Mike 77  
 Shoeb, Mahiba 173, TP120  
 Shoji, Ryo MP191  
 Shorr, Ben WP081  
 Shorr, Benjamin 427  
 Shortelle, Ann RP131  
 Shoulder, Jeanne 544  
 Shoults-Wilson, W TP209  
 Shoults-Wilson, William TP190  
 Shrestha, Babina TP182, TP189  
 Shuang, Chendong TP010  
 Shuang, Chengdong TP011  
 Shuiyuan, Cheng 101  
 Sibley, Paul 619 RP102, TP207  
 Siciliano, Steven MP026, MP098, WP262  
 Siegler, Katie 447, RP107  
 Sigal, Elliot 782  
 Silva, Evelise 53  
 Silva, Flavio MP091  
 Silva, Manori MP119  
 Silva, Thiago RP201  
 Silveira, Cassia MP077  
 Silverman, Keith 129, TP165  
 Sim, Won-Jin TP009  
 Simcik, Matthew 445, TP054  
 Simcox, Alison MP029  
 Simini, Michael TP099, TP104  
 Simmons, Denina 316  
 Simmons, Jane Ellen TP004  
 Simon, Eszter 23, WP075  
 Simon, Stefania RP288  
 Simon-Hettich, Brigitte 129  
 Simoneau, Martha TP245  
 Simonik, Elizabeth RP232  
 Simpson, Andre 324, 766  
 Simpson, Myrna 324, 766  
 Simpson, Stuart WP020  
 Sims, Jerre TP097, MP237, MP239  
 Singer, Joseph TP191  
 Siska, Emily 272, TP204  
 Sitar, Shawn 379, TP013  
 Sitzlar, Megan RP198  
 Sizmur, Tom 31, RP124  
 Sjodin, Andreas 177  
 Skeaff, James 233  
 Skei, Jens 262  
 Skelly, Brian 184  
 Skelly, David WP053  
 Skirrow, Rachel 220  
 Skolness, Sarah MP149, RP222  
 Slattery, Marc TP026  
 Sletta, Haavard 313  
 Sletta, Havard RP094  
 Slobodnik, Jaroslav WP073  
 Slocombe, Andrew MP146  
 Small, Jessica TP165  
 Smalling, Kelly 243, 246, WP054, TP176  
 Smith, Alex WP062  
 Smith, Brendan TP251  
 Smith, Brian WP002, WP068  
 Smith, C RP074  
 Smith, Cassandra RP217  
 Smith, Chris MP134, MP135  
 Smith, Christopher WP155, RP193  
 Smith, Clifford 288  
 Smith, Daniel 241, RP039, WP250  
 Smith, James 32, TP230, RP281  
 Smith, Jeanine 783  
 Smith, John MP177  
 Smith, Kilian TP161  
 Smith, Killian MP121  
 Smith, Loren 248, 249, TP178  
 Smith, Mark WP137, TP187  
 Smith, Philip 538, TP023, TP069  
 Smith, Richard MP029  
 Smith, Robert TP268  
 Smith, Sara 379, TP013  
 Smith, Scott 406, 572, 603, WP206, WP219  
 Smith, Woollcott 591  
 Smyth, Shirley-Ann RP039  
 Snodgrass, Joel WP237  
 Snow, Daniel 159, 305  
 Snow, Nicholas H 360  
 Snyder, Blaine TP225, TP226  
 Snyder, Jennifer RP142  
 Snyder, Shane TP003  
 Snyman, Reineette RP243  
 Soares, Amadeu 323, 415  
 Sobek, Anna 63  
 Soderblum, Erik 218  
 Soeprono, Andrew RP026  
 Soerensen, Anne 35  
 Softcheck, Katherina RP139  
 Sokolova, Inna WP133  
 Solomon, Keith 437, 588 RP102, WP144, RP157, TP207  
 Solorzano-Ochoa, Gustavo RP268  
 Somerwill, Kate 708  
 Sommer, Elizabeth 581, TP240  
 Sommerfield, Christopher TP046  
 Somoza, Gustavo RP263  
 Son, Jino RP059, RP169, WP280, WP284  
 Song, Jeehey WP046  
 Song, Sung Joon TP151  
 Song, Yun RP147  
 Sonne, Christian TP052  
 Sonnemann, Guido RP282  
 Sorell, Tamara MP036  
 Sorensen, Mary 401  
 Sortillon, Don 234  
 Soto, Melina 50, WP135, RP226, RP241, RP280  
 Soubaneh, Youssef RP006  
 Soucek, David RP109  
 Souther, Kitrina TP067  
 Southworth, George RP075  
 Souza, Cristina RP072, MP157  
 Sovic, David RP146  
 Sovocool, Wayne 531  
 Sowa, John 360, RP159  
 Spacil, Michael RP145  
 Spak, Scott 370, 539  
 Sparham, Chris 530  
 Sparling, Donald MP245  
 Sparrevik, Magnus 429  
 Spatz, Dana 575, MP171  
 Spear, Loreal RP016, RP019  
 Spears, Brian 510  
 Spears, Michael 776  
 Spencer, Christine 169, 251, 255  
 Spierre, Susan WP105  
 Splithoff, Henry MP087  
 Sprague, Dan WP261, WP279  
 Sprague, Daniel WP270  
 Springer, Tim 116, TP211  
 Spromberg, Julann RP021  
 Spurgeon, David 323  
 St Clair, Christopher MP247  
 St Louis, Vincent 37  
 Stadler, Teodoro 15  
 Stahl, Leanne TP170, TP171, TP225, TP226, TP260  
 Stahl, Ralph 95, 398, 403, WP192  
 Stalvies, Charlotte 57, TP028, RP041  
 Standley, Laurel WP013  
 Stanier, Charles 539, 551  
 Stanley, J MP241  
 Stanley, Jacob MP240, TP097, TP098, WP159, MP237, MP238, WP166, MP239, WP271, RP081, RP286  
 Stanton, Beckey TP164  
 Stanton, Bruce 465, MP071  
 Stanton, David RP012  
 Staples, Charles TP001  
 Staples, Joseph RP017  
 Stapleton, Heather 166, 177, 179, 454, 459, 464, 546, MP010  
 Stapleton, Patricia TP059  
 Stark, John 486 MP198  
 Starr, James TP138  
 Stauber, Jennifer 403  
 Staveley, Jane 430  
 Stec, David TP268  
 Steeger, Thomas 9, 30  
 Steele, William RP277  
 Steenkeste, Nicolas TP063  
 Steevens, Jeff 734, 736, WP159, WP163, WP166, MP237, MP238, MP239, MP240, RP291  
 Steevens, Jeffrey RP081, MP241, RP286  
 Stefaniak, Sebastian RP126  
 Steffen, Neumann 453, WP073  
 Stegeman, John MP076  
 Steinhoff, Marla 427  
 Stensberg, Matthew WP165  
 Stephens, Jason TP249  
 Stephenson, Gladys MP098  
 Stern, Clay 515  
 Stern, Gary 442, TP144  
 Stern, Jeff MP234  
 Sternberger, Andrea RP172  
 Stevens, Doug RP066  
 Stevens, Jamie MP081  
 Stevenson, Jan WP099  
 Stevenson, Ryan RP137  
 Strickle, William 392.5  
 Stinson, Jonah 104  
 Stir, Ariana RP054, RP288  
 Stiufuc, Rares RP054  
 StLouis, Vincent 255  
 Stoddard, Kati TP271  
 Stoerseth, Trond 313  
 Stoffella, Peter TP068  
 Stojak, Amber 96, MP177  
 Stokland, Oystein 760  
 Stone, Edie MP087  
 Stoner, Kimberly MP067  
 Storseth, Trond 767, 768, RP094, WP140  
 Stout, Dan 72  
 Stout, Scott MP189  
 Strachle, Uwe 333  
 Strahlendorf, Jean WP049  
 Stransky, Chris 730, RP236  
 Strathe, Anders MP159  
 Straus, Dave RP216  
 Streblow, William RP166  
 Strecker, Ruben 329  
 Streissl, Franz 9  
 Stricker, Craig 504

- Strom, Sean 336  
 Strozier, Eric MP020  
 Struger, John TP175  
 Strumpf, Thomas WP021  
 Strynar, Mark TP053  
 Stubblefield, Leslie WP121  
 Stubblefield, William TP022, TP027  
 Stubbs, Chris 785  
 Sturm, Renate MP018, TP040, TP041  
 Stute, Martin 351  
 Su, Guanyong 680  
 Su, Ky TP175  
 Su, Steave 649  
 Suarez, Juan Manuel RP197  
 Subedi, Bikram 60, 716  
 Subramanian, Annamalai 175, 457  
 Subramanian, Mr TP074  
 Sudaryanto, Agus 100, MP124  
 Suedel, Burton 188, 377, RP086, RP089, MP226, WP263  
 Sugatt, Richard WP122  
 Sugaya, Yoshio MP181  
 Suhag, Shivani TP265, WP273  
 Sulecki, Lisa 250  
 Sullivan, James RP074  
 Sullivan, Joseph MP182  
 Sullivan, Julia 261  
 Sullivan, Kristie 121  
 Sulub-Palafox, Yamaral TP255  
 Summers, Heather 104, 108  
 Sumpter, John MP081  
 Sun, Chenfu MP188  
 Sun, Hongwen 368, WP008, TP055  
 Sun, Ping WP125  
 Sun, Yuzhen 677  
 Sunahara, Geoffrey 320, 324, 386  
 Sundaray, Sanjay WP173  
 Sunderland, Elsie 35  
 Sunzenauer, Ingrid MP208  
 Sura, Srinivas RP202  
 Suski, Jamie TP060  
 Suter, Glenn 487, 664, 666  
 Suuberg, Eric TP227  
 Suzuki, Go 175, MP154  
 Suzuki, Toshinari 256, WP150, TP262, TP270  
 Svendsen, Claus 323  
 Swackhamer, Deborah 306  
 Swaddle, John TP172, TP193, WP277  
 Swain, Shella 248  
 Swanson, Heidi RP229  
 Swanson, Wendy 293  
 Swanson, William RP002  
 Swedenborg, Paul 367  
 Sweet, Lauren WP153, WP161  
 Swenberg, James 464  
 Swerhone, George 44, 434  
 Swift, Catherine 511  
 Swigert, James RP138, RP141, RP220  
 Swope, Brandon RP155, WP204  
 Sy, Victoria TP133  
 Sylva, Jason TP203  
 Synowiec, Karen 543  
 Szakal, Christopher WP167  
 Szostek, Bogdan 250  
**T** Tabaran, Flavio RP288  
 Tadele, Kidus 22, 254, 301  
 Taghavy, Amir 735  
 Tagmount, Abderrahmane WP160  
 Tait, Tara 603  
 Takahashi, Hideki TP188  
 Takahashi, Shin 100, 175, 371, 457, 705, TP074, MP154, WP220  
 Takei, Hiroyuki WP162  
 Takeuchi, Ayano MP181  
 Takhar, Mandeep 220  
 Talent, Larry 385, WP048, WP269  
 Tallini, Karin , RP010, MP187, MP213  
 Talukder, Asrar 57, TP028, RP041  
 Tamura, Ikumi WP147  
 Tan, Boon Huan TP063  
 Tan, Shirlee 70, 550  
 Tan, Zhiqiang 268  
 Tanabe, Shinsuke 100, 175, 371, 457, 705, 707, MP007, WP022, WP026, TP061, TP074, MP124, TP141, MP154, WP211 ,  
 Tanaka, Yoshinari 138  
 Tanehill, Lisa WP186  
 Tang, Jianhui TP040  
 Tang, Liang TP162  
 Tanguay, Robert TP107  
 Tanna, Rajiv TP250  
 Tannenbaum, Lawrence 574, 701, WP112  
 Tantra, Ratna 267  
 Tao, Liu MP003  
 Tarrant, Ann 134  
 Tarrant, Darcy 455  
 Tatarazako, Norihisa RP023, WP150, MP181  
 Tatsuta, Haruki 138  
 Tatum-Gibbs, Katoria TP053  
 Taulbee, William WP118  
 Taylor, Allan RP062  
 Taylor, David 595  
 Taylor, Deborah RP062  
 Taylor, Gordon 237  
 Taylor, Lisa 620, MP061  
 Taylor, Robert 512  
 Taylor, Ronald RP038  
 Taylor, Vivien TP243  
 Tazelaar, Dustin MP123, MP201, RP254  
 TBD, TBD 424  
 Tcaciuc, Alexandra 696  
 Tear, Lucinda 232  
 Teed, Scott 484, MP175  
 Teixeira, Camilla 66, 476, MP056, MP057  
 Teixeira, Patricia MP091  
 Tell, Joan TP165  
 Tellini, Karla TP048  
 Tellis, Margaret RP219, WP219  
 Tencic, Adrian MP055  
 Tendler, Brett RP181, RP187  
 Teng, Quincy 223, 765  
 Teo, Yu Ren 356  
 Terasaki, Masanori TP257  
 Teschke, Kay 173  
 Tetreault, Gerald TP130, RP209, TP250  
 Thakali, Sagar 690  
 Thammachoti, Panupong WP051, WP240  
 Thebeau, Nadine 227  
 Theodorakis, Christopher WP139  
 Thibault, Yves WP038, WP039  
 Thienpont, Benedicte TP112  
 Thirakhupt, Kumthorn WP188  
 Thiruvengadathan, Rajagopalan 736  
 Thitiphuree, Tongchai RP095  
 Thoma, Greg RP085  
 Thomas, Johnson 117, MP163  
 Thomas, Linnea 224, 769, MP138, MP149  
 Thome, Jean-Pierre MP150  
 Thompson, Helen 11  
 Thompson, Ian 286  
 Thompson, Tim TP015  
 Thoms, Sharon 79, 82, TP031, MP237, MP238, MP240  
 Thorbek, Pernille 491, 493, WP084, WP086, WP092  
 Thorn, Jonathan 160, TP017, MP020  
 Thorne, Peter 370, 551  
 Thornton, Cammi TP026, MP073, WP163  
 Thorpe, Karen 238, 632, WP028  
 Tidwell, Lane TP246  
 Tiefenthaler, Liesl MP204  
 Tierney, Keith 341  
 Tietge, Joseph 26, 380  
 Tigistu-Sahle, Feven 59  
 Tiller, Brett 290, 296  
 Tillitt, Donald 382, WP115, TP123, RP199  
 Tiwary, Asheesh 543  
 Tjeerdema, Ronald 447, 764, RP107  
 Tkachuk, Alexander 423  
 Tlusty, Michael WP134  
 Tochigi, Saeo TP188  
 Todd, Andrew 405  
 Todd, Charles WP255  
 Todhunter, Brian 420, WP052, MP142  
 Tokai, Akihiro MP228, MP233, MP235  
 Tokunaga, Takahiro TP261  
 Toll, John 478  
 Tollefsen, Knut Erik MP170, RP094  
 Tolson, J Keith MP202  
 Tomasi, Ted MP083  
 Tomasiewicz, Henry WP103  
 Tomasso, Joseph RP216  
 Toms, S MP241  
 Tomy, Gregg 255, MP008, TP054  
 Tondeur, Yves 715  
 Toomey, Rickard RP019  
 Toor, Gurpal 352, 723, RP015  
 Toor, Navdeep 573, WP032  
 Torezani, Evelise WP050  
 Tornier, Ingo 14  
 Torrellas, Marta RP283  
 Torrents, Alba 303, MP096  
 Torres, Leticia RP228  
 Torres Padilla, Juan MP053  
 Tourinho, Paula 323  
 Townsend, David 697  
 Trapp, Crista 297  
 Trawick, Matthew WP058  
 Treguer, Ronan 436  
 Trejo Ramirez, Jose MP053, RP268  
 Trimble, Andrew RP200  
 Tripp, Elizabeth MP131  
 Troiano, Alexandra 51, RP270  
 Trottier, Steve 196, 197  
 Trowbridge, Annette 769, MP138  
 Troy, Jeff RP273  
 Trudeau, Vance TP247  
 Trumble, Stephen 163, MP122  
 Truong, Andrew TP113  
 Trushel, Brittany 21  
 Tsai, Huaijen TP125  
 Tsai, Ying-Tang 236  
 Tsunoda, Shin-ichi TP184, TP185, TP188  
 Tsutsumi, Yasuo TP184, TP185, TP188  
 Tsyusko, Olga TP190  
 Tuberville, Tracey WP267, WP274  
 Tue, Nguyen MP154  
 Tuit, Caroline RP003, RP035  
 Tuit, Carrie TP096  
 Tulve, Nicole TP239  
 Tumber, Vijay 44, 434, WP030, RP202  
 Tumburu, Laxminath WP265, WP268  
 Tunkel, Jay MP217, TP240  
 Tunks, Carolyn 476, MP030  
 Turcotte, Dominique WP030  
 Turner, Andrew 509, MP105  
 Turner, Meredith 218  
 Turnquist, Madeline TP200  
 Turpin, Sean RP153  
 Twardowska, Irena RP126  
 Twiner, Michael WP098  
 Twohig, Marian TP242  
 Tyagi, Tulika WP106  
 Tyczynski, Patrick RP163  
 Tyler, Charles RP012, MP081, WP084  
 Tyler, Heather RP088  
 Tylock, Steven RP118  
 Tyne, William 323  
 Tysklind, Mats 68  
**U** Udaka, Asako TP188  
 Ueda, Norifumi TP141  
 Uehara, Kiriko TP051, WP245  
 Uhler, Allen 73  
 Uji, Miyuki TP188  
 Ulatowski, Gary 728  
 Ulatowsky, Gary TP094  
 Ullman, Jeffrey RP046, RP142  
 Ulrich, Elin 72  
 Ulrich, Nadin WP074  
 Umbstead, Rodney RP183  
 Umbuzeiro, Gisela 148, 151, 152, 154, 155, WP076, RP201  
 Umemura, Takashi TP213  
 Unrein, Julia WP119  
 Unrine, Jason 327, TP190, TP202, TP209  
 Urich, Matthew RP105  
 Usborne, Elizabeth 542  
 Usenko, Sascha 60, 163, 716, MP114, MP122  
**V** Vaissiere, Bernard 10  
 Vale, Carlos 289, MP027, MP028, TP253  
 Valenti, Ted RP103  
 Valenti, Theodore 618, RP100, RP110, RP266  
 Valentine, Marla 114  
 Valverde, James RP086  
 Valverde, L 1  
 Vamshi, Raghu WP014  
 van Aggelen, Graham 220  
 van Bergen, Saskia 166  
 van den Brink, Paul 401  
 Van den Eede, Nele 178, TP142  
 van den Heuvel, Michael 567, WP036, MP209  
 Van den Hurk, Peter RP189  
 van der Heijden, Stephan WP069  
 Van Der Kraak, Glen WP033, WP036, MP140  
 van Egmond, Roger 530, RP029  
 Van Gaal, Luc 374, TP143  
 van Geen, Alexander 351  
 Van Geest, Jordana 615, TP207  
 Van Genderen, Eric 226  
 Van Gestel, Cornelis 323  
 Van Ginneken, Inge 126  
 Van Metre, Peter 366, 439, TP156  
 Van Scoy, April 764  
 Van Sprang, Patrick 231, RP227  
 Van Tiem, Lindsey 564  
 Van Veld, Peter 17  
 van Vuren, J.H.J. MP080  
 Van Wensem, Joke 746  
 Van Wyk, Mia MP196  
 Vandenheuvel, Michael 238, 566, WP033, WP037  
 VanDenHeuvel, William WP113  
 Vandenhove, Hildegarde 496  
 Vander Pol, Stacy TP237  
 Vanrolleghem, Peter WP082  
 VanTiem, Lindsey TP125  
 Varanusupakul, Pakorn WP051, RP095, RP099  
 Vardy, David RP181  
 Varughese, Eunice TP004  
 Vassilenko, Ekaterina RP093  
 Vasudevan, Dharni 658, WP247  
 Vaughan, Mace 10  
 Vecities, Chad 626  
 Veldhoen, Nik 220  
 Veldhuis, Linda WP145  
 Velinsky, David 83, TP046  
 Velleux, Mark MP229  
 Venier, Marta 536, MP013  
 Venners, Scott TP121  
 Verbeck, Guido 32  
 Verbrugge, Lori 495  
 Verdonck, Frederik WP125  
 Verkoeyen, Stephanie 556, 557, MP155, WP180  
 Verreault, Jonathan MP009, MP014, MP016  
 Verslycke, Tim 430, 526, 644, 690  
 Verta, Matti TP064, TP077  
 Verwiel, Ann WP244  
 Vesper, Stephen TP004  
 Vey, Matthias WP016  
 Viant, Mark 225  
 Vicent, Teresa 532  
 Vieira, Fernanda 712  
 Viet, Pham 457, 705  
 Vignati, Davide TP076  
 Vigneault, Bernard WP205  
 Vigo, Craig TP005  
 Vigon, Bruce 109, 110, RP282  
 Villalba, Maria 350  
 Villegas, Eric TP004  
 Villeneuve, Daniel 29, 218, 223, 224, 375, 558, 679, 769, MP138, MP149, MP151, MP152, TP134, RP038, RP090, RP151, RP222  
 Vinas, Rene RP148  
 Vinciotti, Veronica MP081  
 Vining, Bryan 715  
 Vogel, John 1, RP086



## AUTHOR INDEX

- Vogel, Timothy TP044  
Vogel II, John 425, RP089  
Vogt, Sarah RP173  
Volz, David 375  
von Hippel, Frank 383  
Vonderohe, Peter 453  
Vongphachan, Viengtha 338  
Voorhees, Jennifer 447, 764, RP107  
Vorhees, Donna MP193  
Voros, Craig WP118  
Voutchkova, Adelina RP053  
Vukanti, Raja 322  
Vulpe, Chris 451, RP038, WP101, WP102, TP128, WP160, TP187  
Vuori, Kari-Matti RP213  
**W** Wacksman, Mitch 80  
Wada, Naoki MP233  
Wagbo, Ane 565  
Wagener, Angela TP048  
Wages, Mike RP271  
Waghiyi, Vi 383  
Wagman, Michael 575, MP171  
Waiser, Marley 44, 434, RP202  
Waites, Crystal RP193  
Waits, Eric 462, 630  
Wakefield, Jeffrey TP038, WP109  
Walker, John RP183  
Walker, Virginia 580, 628  
Wall, Steven WP091, MP178, RP252  
Wallace, Gordon 596, 699, 700, RP074  
Walsh, Elizabeth RP171, RP225  
Walsh, Jim 81  
Walter-Rhode, Susanne 329  
Walters, David 98, 193, 504, WP027, RP170  
Walters, Lynn TP226  
Wan, Yi RP063  
Wang, Bo MP174  
Wang, Daisy 5  
Wang, Dongli 369, TP139  
Wang, Feiyue 283, MP158  
Wang, Hailin TP124  
Wang, Jia TP078, TP079  
Wang, Jingxin 572  
Wang, Jinyuan MP019  
Wang, Jinyuan RP196  
Wang, JiZhong 260  
Wang, Joy TP241  
Wang, Lei WP008  
Wang, Lixin RP007  
Wang, Magnus WP089  
Wang, Nan 471, 568  
Wang, Ning 250, MP039, TP055, RP178, WP224, RP249  
Wang, Pu 259  
Wang, Qianheng 72  
Wang, Rong-Lin 558  
WANG, Rui MP034  
Wang, Tiewu 105, RP063  
Wang, Wen-Xiong 312, MP034  
Wang, Xiao MP005  
Wang, Xiaowa 476, MP030, MP032, MP056, TP087  
Wang, Xiaowei MP011  
Wang, Yonggang 735  
Wang, Yuan WP031  
Wang, Zaosheng WP071  
Wang, Zhendi WP190  
Wang, Zhixin TP124  
Wania, Frank 442, 642, 689, MP004  
Wannamaker, Eric RP035  
Wanty, Richard 504  
Warby, Richard MP118  
Ward, Joshua 83  
Ware, Michael TP004  
Waria, Manmeet 352  
Warner, Chris 633  
Warner, Kara MP168  
Warner, Sarah WP187  
Warren-Hicks, Bill 11  
Wartian, Matthew 743  
Washburn, Kate 145  
Washington, John RP162  
Watanabe, Haruna RP023, WP150  
Watanabe, Karen 679  
Watanabe, Mafumi TP061, TP141  
Waters, Katrina WP068  
Wathen, John TP170, TP225, TP260  
Watkins, Andrew TP053  
Watkins, Deborah 179  
Watson, AtLee RP245  
Watson, Cheryl RP148  
Watson, Gary 189  
Watson, Richard 753  
Watts, Alison 362  
Watts, Michael RP124  
Watts, Richard RP046  
Wautier, Kerry RP253  
Wayland, Mark 67  
Weaver, Alissa 480  
Weaver, Paul MP041  
Weavers, Linda 545  
Webb, Diane 49, MP050, RP258  
Webb, Elyn 160  
Webb, Sarah 416  
Webb-Turbeville, Jayme TP170, TP171  
Weber, Daniel 381, WP103, TP107  
Weber, Gregory 524  
Weber, Roland 720  
Webster, Glenys 173, TP058, TP121  
Webster, Thomas 166, 177, 179, MP127  
Wehmas, Leah MP149, RP151  
Wei, Alex WP165  
Wei, Gaoling MP022  
Wei, Qingshan WP165  
Wei, Zhang RP077  
Wei-Haas, Maya TP238  
Weigel, Stefan 266  
Weinberg, Howard 348  
Weiner, Barbara MP021  
Weinstein, John 692  
Weir, Scott WP048, WP059  
Weiss, Charles 736  
Weiss, Jana 23  
Weiss, Jeri MP029  
Welch, Linda WP255  
Wells, Randall TP195  
Wenning, Rick 671  
Wenxiong, Wang RP077  
Wepener, Victor MP080  
Werner, David 92  
Werner, Inge 318  
Werner, Jeffrey 710  
Wesoloh, Chip 167, 257  
West, Ashley RP019, MP129, MP136  
West, Carol RP074  
West, Charles 570  
westerhoff, paul 270  
Westfall, Josh RP153  
Westgate, Andrew WP110  
Weston, Donald 88  
Wetzel, Dana 525  
Whalen, Joann 320, 324  
Whaley-Martin, Kelly WP193  
Whatling, Paul 119, MP169, MP184  
Wheatcraft, Stephen 645  
Wheeler, Amanda MP155  
Wheeler, James WP084  
Whicker, Jeffrey 4  
Whitaker, Brian WP227  
White, Ariel WP251  
White, Jason 321  
White, Joseph 127  
White, Lori ,TP111, TP113, TP131, RP165, WP201  
White, Sarah 42, 598, MP218  
Whitehead, Andrew 135, 392.5  
Whitfield Aslund, Melissa 324, 766  
Whiting, Ashley RP125  
Whitley, Annie 327  
Whitney, Margaret TP172  
Wiberg, Karin 68  
Wichmann, Arne 334  
Wickliffe, Jeffrey 779  
Wicklum, Dan 473  
Wickwire, Ted MP222  
Wickwire, Theodore MP167  
Widmeyer, Joline 576  
Wiechmann, Mark 423  
Wiessler, Manfred 586  
Wiggins, Sandy 416, 562, WP049  
Wigginton, Andrew RP183, RP278  
Wilbanks, Mitchell 385, 633, TP132, RP256  
Wilcox Freeburg, Eric WP134  
Wild, Bill 384  
Wilde, Susan WP097  
Wildhaber, Mark 382  
Wilhelm, Steven WP098  
Willett, Catherine 121  
Willett, Kristie TP026, MP073, WP163  
Williams, Bethany 421, MP038  
Williams, Clayton 627  
Williams, E 366, TP156, WP156  
Williams, Geoff MP068  
Williams, Jamma MP237, MP239, RP291  
Williams, Kate MP029  
Williams, Larissa 463  
Williams, Les 290  
Williams, Lisa WP113  
Williams, Mary TP249  
Williams, Rebecca MP079  
Williams, Richard 582  
Williams, Susan 701, WP112  
Williams, Tony WP272  
Williams, Travis 582  
Williamson, Mary 251  
Willis, Theodore RP017  
Willming, Morgan RP210  
Wilson, Christopher 352  
Wilson, Eric RP132  
Wilson, Glenn TP246, TP248  
Wilson, Jennifer 439  
Wilson, Karen RP017  
Wilson, Kenneth WP035  
Wilson, Natalie 80  
Wilson, Patrick 726, TP068  
Wilson, Vickie 17, TP004, TP007  
Winchell, Michael MP184  
Winding, Anne WP070  
Winesett, Steve RP198  
Winkelman, Dana 431  
Winkens, Kerstin 711  
Wirgin, Isaac 52, 466, 467  
Wirzbicki, Kathryn TP232  
Wise, Stephen TP237  
Wiseman, Clare 600  
Wiseman, Steve 471, 568 RP187, RP204  
Wisk, Joseph 10, MP182  
Witt, Melanie 285  
Wiwchar, Logan 341  
Wolf, Cindy TP053  
Wolf, Craig 275  
Wolf, Steven WP094  
Wolf, Thomas MP169  
Wolfe, Daniel TP008, TP168, RP238  
Wollenweber, Marc 711  
Wolstenholme, Barry TP055  
Wolter, Faren 156  
Won, Eun-Ji 139, 561, 563  
Wong, Charles WP004  
Wong, Diana RP138, RP141, RP220  
Wong, Fiona 442, TP144  
Wong Chang, Irma MP190  
Wood, Chris 406, 506, 507, MP040, WP195, WP207, WP210, RP219, WP225  
Wood, Christopher 603, MP046, MP047, MP049, WP219  
Woodard, Valerie 575, MP171  
Woodburn, Kent 99, TP081, RP182, RP194  
Woods, Tim 81  
Woodward, Allan 752  
Woodworth, Adam WP044  
Wooster, David RP142  
Word, Jack 608  
Woudneh, Million TP056, TP066, TP119  
Wray, Austin TP065  
Wrench, Nicola TP007  
Wright, Demitria 292  
Wright, Raymond 414, WP047  
Wrona, Fred 473, 474, MP033  
Wu, Dongmei 719  
Wu, Jun 726, TP068  
Wu, Nerissa MP127  
Wu, Qian 372, WP011  
Wyn, Brianna 33  
**X** Xenopoulos, Marguerite 45, 627  
Xia, Kang 396  
Xia, Xiangsheng 380  
Xia, Xiaoyan 164, 165, MP058, RP064  
Xie, Qing 684  
Xie, Zhiyong MP018, TP040, TP041  
Ximba, Bhekumusa RP243  
Xiurui, Guo 101  
Xu, Shi MP094  
Xu, Shihe 540, WP009, RP047, TP145  
Xu, Ting MP120  
Xue, Yuzhi TP010  
**Y** Yabe, John TP213  
Yacoob, Sumera WP232  
Yaguchi, Kumiko 256, TP262  
Yamada, Tadasu WP026  
Yamada, Yuki RP096  
Yamamoto, Hiroshi WP147  
Yamashita, Kohei TP184, TP185  
Yan, Fei MP231  
Yan, Norman WP206  
Yanagimoto, Hayato , TP061, TP141  
Yang, Chun WP190  
Yang, Fan MP030, MP117  
Yang, Jae-Ho TP141  
Yang, Sarah WP169  
Yang, Song Yi WP173  
Yang, Xiaoguang RP114  
Yang, Xinyu RP292  
Yang, Zeyu WP190  
Yano, Miho TP270  
Yano, Natalia 408, WP202  
Yao, Satoshi MP228  
Yasuda, Yusuke WP147  
Yasugi, Shin-ya 707  
Yates, Brian WP096, RP276  
Yates, Dave 517  
Yates, Michael TP025  
Yauk, Carole 338  
Ye, Feng 256  
Yee, Don MP192  
Yeung, Leo MP021, RP067  
Yi, Jongheop RP290  
Yi, JongHeop RP078  
Yi, Seung-Muk TP199  
YI, Zhongsheng 677  
Yilmaz, Vedat TP073  
Yim, Byung-Jin MP088  
Yim, Un Hyuk 107, RP004, TP042, TP269  
Yim, Un Hyuk Yim 107  
Yim, UnHyuk RP034  
Yin, Daqiang MP120, RP265  
Yin, Yongguang MP011  
Yingling, Hannah 575, MP171  
Yokel, Jerry TP174  
Yonkos, Lance 17  
Yoon, Sung-Ji RP203  
Yordy, Jennifer TP075  
York, Duncan MP164  
Yoshida, Tokuyuki TP188  
Yoshikawa, Tomoaki TP184, TP185, TP188  
Yoshioka, Yasuo TP184, TP185, TP188  
Yost, Christopher RP045  
Yost, Erin MP137  
Yost, Lisa 722  
You, Jing 87, WP065, WP066, MP099, MP131, RP221  
Young, Brian RP263  
Young, Rozlyn WP037  
Young, Suzanne 96, MP177  
Young, Teresa MP056  
Yu, Hongxia 680  
Yu, Huan-Yun TP050  
Yu, Seung Ho WP152  
Yu, Shaiguang 624  
Yu, Shen 604  
Yu, Shuangying RP271  
Yu, Sujuan 268

- Yucuis, Rachel 541  
 Yun, Hyo-In MP103, MP115  
 Yun, Sehun RP062  
**Z** Zabik, Jack 722  
 Zaharie, Florin RP054  
 Zahlsen, Kolbjoern 313  
 Zajdlik, Barry MP098  
 Zandpoor, Golnar 265  
 Zandpour, Golnar TP049  
 Zanette, Juliano 53, MP076, MP077  
 zaroni, maria valnice 151  
 Zaoudeh, Linda 83  
 Zapata-Perez, Omar RP289  
 Zaragoza, Eliza TP217  
 Zaroogian, Gerald TP245  
 Zaugg, Steven 441  
 Zavodnik, Shane WP055  
 Zeeb, Barbara MP132  
 Zeigler, Christian 646  
 Zeise, Lauren 402  
 Zemba, Stephen 7  
 Zemo, Dawn 543  
 Zeng, Eddy 260, MP022, TP050, MP094  
 Zhang, Aiqian 677  
 Zhang, Baohong 521, WP012  
 Zhang, Baozhong 87  
 Zhang, Carl TP016, TP018  
 Zhang, Changwen RP007  
 Zhang, Gan TP040, TP146  
 Zhang, Hongchang MP120  
 Zhang, Hui 158, 264, 356, TP063  
 Zhang, Jianshun WP191  
 Zhang, Jianying WP099  
 Zhang, Lin 69, 654  
 Zhang, Mancheng TP011, TP012  
 Zhang, Qinghua 259  
 Zhang, Shuping RP007  
 Zhang, Siyu 684  
 Zhang, Tao 368, WP008  
 Zhang, Tina 658  
 Zhang, Xianzhong WP008  
 Zhang, Xiaowei 680, WP157  
 Zhang, Yanqiong WP012  
 Zhao, Heidy WP106  
 Zhao, Jiujiang MP117  
 Zhao, Lijie WP008, TP055  
 Zhao, Yun RP008  
 Zhao, Zhen TP041  
 Zheng, Lei 664, RP262  
 Zheng, Li 659  
 Zheng, Yan 351  
 Zhong, Guangcai TP040  
 Zhou, Jing 684  
 Zhou, Jizhong 215  
 Zhou, Meng 697  
 Zhou, Qing TP011, TP012, MP116  
 Zhu, Jiping WP191  
 Zhu, Yi-Ping RP008  
 Ziegler, Gregory RP025  
 Ziegler, Kirk 659  
 Zimmer, Alex WP195  
 Zimmer, Elke 489  
 Zimmerman, Julie RP053  
 Zimmerman, Marc 432  
 Zintek, Larry 298  
 Ziv, Lisa TP165  
 Zoeller, Thomas 343  
 Zoh, Kyung-Duk MP145, TP199  
 Zou, Enmin RP224, RP240  
 Zou, Xiaoming RP265  
 Zucollo, Guilherme 151  
 Zuellig, Robert 504, 663  
 Zuin, Lucia 573  
 Zulkosky, Ann 694  
 Zuniga-Bermudez, Gerardo TP179  
 Zushi, Yasuyuki 256  
 Zwiernik, Matthew 27, 719, MP035, MP123, MP180, MP201  
 Zychowski, Gregory 416, WP049

- A** Aarhus University 89, 180, 205, 375, WP067, WP070, MP121, TP218  
Aarhus University, Faculty of Agricultural Sciences MP121  
Aarhus University, National Environmental Research Institute 89, MP121, TP161  
ABC Laboratories RP264  
Abo Akademi University RP044, TP062  
Academy of Natural Sciences 83, TP046  
Acadia University 31, 33, MP026, MP027, MP032, RP130  
Advanced Industrial Science and Technology WP209, RP251  
Advanced Materials Research Center 350  
AECOM 359, 591, 693  
Agency for the Assessment & Application of Technology 100  
Agriculture and Agri-Food Canada MP169, RP283  
Agriguard Company, LLC WP259  
Agroscope Changins-Wädenswil 773  
Agroscope Liebefeld-Posieux Alp 11  
Akdeniz University WP158  
Akvaplan-niva 606  
Al. I. Cuza University of Iasi TP142  
Alaska Community Action on Toxics 383  
Alaska Department of Environmental Conservation 495  
Alberta Environment 473  
All North Consultants MP037  
Alliance for Environmental Technology MP222  
Alpha Analytical RP193  
ALS Laboratory Group 174  
Alterra Wageningen University and Research Centre 401, MP060, RP133  
AMEC 554, 686, 730, MP006, MP036, WP 121, WP126, WP127, WP241, WP244, RP131, RP236  
American Bird Conservancy 9  
American Chemistry Council 123, TP001  
American Petroleum Institute RP138, RP141, RP220  
Analytical Perspectives 715  
Analytisch-Biologisches Forschungslabor GmbH TP137  
Anchor QEA, LLC 189, 659, TP031, TP083, RP048  
Applied Pharmacology & Toxicology, Inc. 118, 123, 332  
Aqualytical Services Incorporated TP037, MP111  
Aquatic Ecology and Toxicology Group 333  
ARC Arnot Research & Consulting Inc. 208  
Arcadis 2, 77, 78, 79, 80, 82, 84, 96, 127, 130, 190, 364, 389, 390, 413, 479, 480, 731, 781, 783, 784 MP085, RP111, WP126, MP139, TP150, TP155, TP169, MP177, MP179, MP220, MP224, MP225  
Arcadis Belgium RP227  
ArcelorMittal Tubarão WP050, TP214  
Archbold Biological Station 247  
ARCHE 231, WP125, RP227  
Ardea Consulting MP182  
Arizona State University 270, 624, 714, MP118, WP105, WP123, RP118, RP267  
Asbury University RP183, RP278  
Ashland University RP200  
Assessment Technologies, Inc. TP001  
Assistant Professor WP045, WP059, TP060, WP093, MP141  
Assiut University MP127  
Associated Engineering Alberta Limited RP039  
Assumption College TP232  
Astra Zeneca 129  
Astrix 160  
Atlantic PIRI (Partnership in Risk Based Corrective Action) 191  
Atomic Energy of Canada Ltd. 497  
Avatar Environmental, LLC 81  
Avian Conservation Center and Center for Birds of Prey TP075  
AXYS Analytical Services 255, 571, TP056, TP066, TP118, TP119  
Azimuth Consulting Group Inc. 189
- B** Badger Technical Services RP038, TP097, WP159, WP166, MP237, TP014, TP103, WP249, WP263, RP287  
Bahme & Associates 645  
Barbara J. Goldsmith & Company 424  
Barnard College 351  
Baruch College  
BASF 10, 123, 129, MP162, MP182, WP257, RP133  
Battelle 160, 347, 590, 591, 611, 693, 695, 697, 699, 700, TP017, MP020, WP013  
Battelle Pacific NW Division-MSL 102  
Bayer CropScience 9, 10, 15, 16, 118, 120, 123, 332, 492, 724, 725, MP063, MP068, MP161, MP206, WP257, WP275  
Baylor University 46, 60, 163, 366, 430, 716, RP030, RP053, WP095, MP114, MP122, WP156, TP156, TP210, TP215  
BEC Technologies, Inc. TP001  
Bechtel Jacobs RP075  
Bee Alert Technologies, Inc 16  
Beijing University of Technology 101, MP005, RP114  
Benedict College 692  
Bermuda Institute of Ocean Science 656  
Bermuda Zoological Society 420, WP052  
BioDetection Systems 23, WP075  
Biodiversity Research Institute 31, 512, 517, TP025, MP029, TP105, WP111, RP130, TP191, TP200, WP255  
Bioforsk 262  
BioMedware, Inc MP216  
Biotechnology Research Institute 386  
BioTriX 313, 609, 767, RP094, WP140  
Black & Veatch Special Projects Corp WP236  
Blanchard River Watershedpartnership WP100  
Blasland Bouck and Lee 77, 78  
Boston University 54  
Boston University School of Public Health 166, 177, 179, MP127  
Bowdoin College 607, 658, WP110, WP247  
BP 210, 211, 217, TP027, TP035, TP036, TP224  
Braskem 712  
Bridgestone Americas, Inc. 483, 785, MP130  
Bridgewater State University RP275  
British American Tobacco, R&D Center TP137  
British Geological Survey RP124  
Brock University MP008  
Brooks Rand Labs  
Brown and Caldwell MP133  
Brown University 135, 145, 261, 446, 452, WP020, TP227  
Browning's Honey Company, Inc. 10  
Brunel University MP081  
BTS 377, 633, RP090, TP134  
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit 9  
Busan Metropolitan City Institute of Health and Environment 440
- C** CI Agent Solutions TP037  
California Agricultural Research, Inc. 10  
California Department of Fish and Game TP164  
California Department of Pesticide Regulation 10, RP027  
California Department of Public Health 369, MP127, TP139  
California EPA 402, 455, 456, WP025  
Calvatis GmbH 586  
Calvin College 513, WP113, TP191  
Cambridge Environmental Inc. 7, 720  
Campeche Autonomous University/EPOMEX Center 258, WP246, TP255  
Canadian Light Source Inc. 44, 573, WP032, WP035  
Canadian Rivers Institute, University of New Brunswick 33, 251, 637, MP032, TP081, TP093, TP194, RP229, RP253  
Canadian Rivers Institute, University of Prince Edward Island 238, 566, 567, 632, WP028, WP033, WP036, WP037  
Canadian Wildlife Service 167  
CANMET-MMSL 233, RP070, RP071  
Cape Canaveral Scientific, Inc. TP196  
Cape Peninsula University of Technology WP005, WP006, RP243  
Carbolytic Materials Company RP117  
Cardiff University RP012  
Cardno Entrix 395, 397, 599, 602, TP015, TP022, TP027, TP038, MP083, MP123, WP109, RP136, MP180, MP201, RP223, TP224, WP253, RP254, WP266  
Carleton University 167, 257, 338, 565, MP009, MP012, MP014, MP015, MP016, TP052, WP272  
Carnegie Mellon University 5, 428, WP069  
Cary Institute for Ecosystem Studies RP170  
Catholic University of Daegu TP141  
CCA-UFSCar 11  
CDM 365, RP002, TP158, TP160  
CE2 Consulting WP019, TP085  
CEF, Faculty of Pharmacy, University of Coimbra, Coimbra TP166  
Cefas MP166  
Cemagref 202  
Cenibark International, Inc. 291  
Center for Disease Control and Prevention 177  
Center for Environmental Science in Saitama 256, RP060, TP148, TP162  
Center for Green Chemistry and Green Engineering at Yale 584  
Center for Research on Women's and Children's Health 455  
Center for Risk Science Innovation and Application, ILSI Research Foundation 269  
Center of Environmental Implications of Nano Technology (CEINT) WP161  
Centers for Disease Control and Prevention MP119  
Central Methodist University RP143  
Central Military Hospital 108 705  
Central Science Laboratory 11, 433  
Centre for Ecology and Hydrology RP012  
Centro de Investigaciones Biológicas del Noroeste, S.C. TP217  
Centro de Investigaciones del Medio Ambiente – UNLP, CONICET RP032, TP133, RP263  
Centro para el Estudio del Agua WP135, RP280  
Centro Tecnológico AGQ América RP197  
CERMN, University of Caen MP001  
CETESB – Companhia Ambiental do Estado de São Paulo WP243  
CH2M Hill 291, 292, 294, 670, 749, MP134, MP135, TP173, TP206, MP212, WP266, RP254  
CH2M HILL Canada 56  
CH2M HILL Plateau Remediation Company 291, 292, TP173  
Chatham University 443  
Chemical & Biological Engineering RP093  
Chemicals Regulation Directorate 490, MP195  
Cheminova, Inc. 119, MP169, MP184  
ChemRisk MP146, MP221  
Chevron Environmental Technology Company 543  
Chia Nan University of Pharmacy and Science RP175  
Chicago Zoological Society, Mote Marine Laboratory TP195  
Chihuahua Autonoma University 350  
Chinese Academy of Sciences 105, 677, TP124, RP063  
Chinese Academy of Sciences, Institute of Urban Environment 604  
Chinese Academy of Sciences, Research Center for Eco-Environmental Sciences 105, 259, 268, 677, MP005, MP011  
Chinese Academy of Sciences, South China Sea Institute of Oceanology RP077  
Chinese Academy of Sciences, State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences RP007  
Chinese Academy of Sciences, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry 87, TP146, WP065, WP066, MP099, MP131  
Chonbuk National University TP126, WP141  
Chonnam National University WP173, MP194, MP211  
Chulalongkorn University WP051, RP095, RP099, WP188, WP240  
Chungnam National University, College of Agriculture & Life Science, Department of Applied Biology & Chemistry MP103, MP115  
Chungnam National University, College of Veterinary Medicine MP103, MP115  
CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos 415  
Cinvestav Unidad Merida 258, RP230, MP246, RP289  
CIRAIG 706, 775, RP116, RP119  
Citizen's Institute for Environmental Studies TP151  
City of Calgary RP039  
City of San Diego 279  
Clarkson University 164, 165, 445, 392.5, MP054, MP058, RP064  
Clemson University 42, 98, 140, 162, 235, 307, 308, 376, 623, 738, WP010, WP018, WP027, , TP065, RP081, MP104, RP145, RP147, WP149, WP153, WP161, RP081, RP164, RP189, MP218, RP286  
CLF-Chem Consulting TP057  
CNRS 202, 468  
Coastal Monitoring Associates MP134, MP135  
Colgate University 460  
Colgate-Palmolive 529  
College of Marine and Earth Studies, University of Delaware TP046  
College of Staten Island  
College of William & Mary 400, 512, 517, TP172, WP111, WP251  
College of Wooster 357, RP011  
Colorado School of Mines 270, 353, 413, 640 TP122, TP201  
Colorado State University 235, 401, 431, 663, 668, RP172, WP285  
Columbia Analytical Services TP205  
Columbia University 455, 535  
Compliance Services International 119, MP059, MP205, MP206



Computer Sciences Corporation 380  
 Concurrent Technologies Corporation 778  
 Conestoga-Rovers & Associates TP153, WP250, RP061  
 Conicet, Centro de Investigaciones del Medio Ambiente, Facultad de Ciencias Exactas, Universidad Nacional de la Plata RP032, TP133, RP263  
 Connecticut Agricultural Experiment Station 321, 657, MP067  
 Consultant 11  
 Conte Anadromous Fish Research Center, USGS 523  
 Cooperative Institute for Limnology and Ecosystems Research 379  
 Copper Development Association 413, RP264  
 Cornell University 337, 710, MP087  
 Cornell University, Department of Biological and Environmental Engineering 711  
 Cranfield University 712  
 CropLife America 118, 245, 332  
 CropLife Foundation 245  
 CSC MP216, TP225, TP226  
 CSIC 717, TP112  
 CSIR, Water Research Institute 371  
 CSIRO 57, 403, WP020, TP028, RP041  
 CSX Transportation 781  
 Currenta TP001  
 Curtin University 49, 516, RP008, RP014, MP050, WP108, RP258  
**D** Dalhousie University MP068  
 Dalian University of Technology 172, 684  
 Dartmouth College 146, 465, WP208, TP243  
 Dartmouth Medical School 465, MP071  
 Dauphin Island Sea Lab TP026  
 Decision Partners 423  
 Defense Threat Reduction Agency 386  
 Delaware Department of Natural Resources and Environmental Control TP046  
 Delaware River Basin Commission RP028, TP046  
 Denmark Technical University WP200  
 Department of Civil & Env Engineering 621, 625, 626, TP135  
 Department of Defense MP217  
 Department of Fisheries and Oceans Canada 227, 255, 265, TP049, RP253  
 Department of Water, Government of Western Australia RP014  
 DePauw University, Department of Biochemistry MP149  
 Dermatopathology Associates 32  
 Desert Research Institute 645  
 Deutsches Krebsforschungszentrum 586  
 Dhaka University 351  
 Diamond V RP145  
 Diavik Diamond Mine Inc. RP137  
 District of Columbia Water and Sewer Authority 303, MP096  
 Dow Agrosciences 9, 117, 332, MP062, MP064, MP065, RP087, MP163  
 Dow Chemical Company 117, 123, 722, MP163, TP001, RP139  
 Dow Corning Corporation 99, 540, WP009, MP035, RP047, TP081, TP085, TP145, RP182, RP194, MP201, TP231, TP236  
 Dowling College 580, 628, RP082  
 DSO National Laboratories TP063  
 Dublin City University 688, 691, 709, RP055, RP057, RP058, TP219, TP220, TP221  
 Duke University 46, 133, 145, 153, 166, 177, 179, 218, 271, 274, 394, 400, 454, 459, 464, 546, 564, 694, 732, MP010, RP013, MP024, MP111, TP125, MP137, TP202, MP203, RP245, RP292  
 DuPont 95, 123, 398, 403, 613, TP057, WP192, WP257  
 DuPont de Nemours Deutschland 11  
 DuPont, Haskell Global Centers 218, TP181  
 Durham University 682  
 Dynamac Corporation TP170, TP171, WP265, WP268  
 Dynamic, Inc WP281  
**E** E-parisara Ltd 457  
 E.I. du Pont de Nemours & Company 250, 724, TP055, TP057  
 ENCB IPN TP179  
 E2 Consulting Engineers, Inc. WP091  
 EA Engineering, Science and Technology, Inc. 109, 110, 281  
 Earthspan TP025  
 East Bay Municipal Utility District 166  
 East Carolina University 521, WP012  
 Eawag/EPFL, Swiss Centre for Applied Ecotoxicology 318  
 Eawag, Swiss Federal Institute of Aquatic Science and Technology 453, 460, WP073

EBA A Tetra Tech Company 576  
 EcoBenefits Research 745  
 Ecole Centrale de Lyon TP044  
 Ecole des Hautes Etudes en Santé Publique RP056  
 Ecole Polytechnique de Montreal 706, 775  
 Ecological Risk Inc. 291, 292, 294, WP093, TP173  
 Ecological Society of America 740  
 EcoSafety Sciences WP017  
 Ecosystem Management & Associates, Inc. 210, 392  
 Ecosystems Research Group, Ltd MP029  
 EcoTox Assessments, LLC RP138, RP141, RP220  
 Edgewood Chemical Biological Center 386, TP099  
 Edson Ecosystems LLC 750  
 EFSA (European Food Safety Authority) 9  
 Ehime University 100, 175, 371, 457, 705, MP007, WP022, WP026, TP061, TP074, TP141, MP154, WP211, WP220  
 Ehime University, Center for Marine Environmental Studies 175, 707, MP124, WP022  
 Ehime University, Senior Research Fellow Center 175, MP124  
 EHS Support Corporation MP215  
 Electric Power Research Institute 284  
 Eli Lilly and Company TP169  
 EMPA 737  
 EMR RP266  
 Energy Answers International 783  
 ENTRIX, Inc. TP228  
 ENVIRON 284, 401, 483, 529, 647, 671, 672, 781, 785, TP057, TP157, WP062, WP063, WP064, RP069, RP087, MP130, RP140, RP149, RP160, WP260  
 Environment Agency MP081  
 Environment Canada 27, 33, 37, 62, 65, 66, 67, 97, 149, 150, 167, 169, 171, 173, 191, 195, 196, 197, 198, 199, 200, 209, 235, 251, 252, 255, 257, 263, 316, 328, 338, 380, 399, 442, 470, 473, 474, 475, 476, 477, 484, 518, 556, 557, 565, 569, 573, 577, 616, 620, 641, 665, 672, 719, 747, 748, 750  
 MP008, MP014, MP015, MP016, MP026, MP027, MP030, WP030, MP031, WP032, MP032, TP033, WP034, WP035, RP036, RP037, RP039, WP044, TP054, MP056, MP057, TP058, MP061, MP070, WP077, TP081, TP085, TP087, RP091, MP117, TP120, TP130, MP144, TP144, MP155, TP175, WP190, TP194, MP200, RP202, RP209, TP250, WP272  
 Environment Canada, Canada Center for Inland Islands MP040, WP207  
 Environment Canada, National Wildlife Research Centre MP009, MP012  
 Environment Canada, Pacific Environmental Sciences Centre 220  
 Environment Canada, Water and Climate Impact Research Centre MP033  
 Environment Canada, Water Science and Technology Directorate 44, 434, TP058, WP035, RP202  
 Environmental & Turf Services, Inc. 645  
 Environmental and Regulatory Resources 123, 332, RP193  
 Environmental Assessment Services 290, 296  
 Environmental Chemistry Laboratory WP025  
 Environmental Consultant 114  
 Environmental Health National Program 665, RP045  
 Environmental Institute WP073  
 Environmental Protection Agency 371  
 Environmental Resource Consultants 752, MP043, RP177, RP212  
 Environmental Resource Management RP024, MP189, MP227  
 Environmental Science & Green Chemistry Consulting, LLC 582  
 Environmental Sciences Group, Royal Military College of Canada RP127  
 Environmental Toxicology Research Centre MP009, MP014, MP016  
 EPA National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division RP038  
 EPOMEX, Universidad Autonoma de Campeche 258  
 Equilibrium Environmental Inc. RP121, TP154  
 ERDC-EL-EP-P 377, 633, TP132, RP256  
 ERM 186, RP024, MP185, MP189  
 Escuela Nacional de Ciencias Biológicas, IPN TP179  
 Estonian University of Life Sciences TP070  
 ETH Zurich 373, 399, 653, TP087  
 Eurofins Agroscience Services 14  
 Eurofins-GAB GmbH 14  
 European Commission, Joint Research Centre, ECVAM 329, TP085  
 Evonik Goldschmidt GmbH RP047  
 Exova RP121

Exponent Inc. 80, 119, 123, 192, 210, 211, 213, 214, 332, 361, 403, 430, 481, 543, 594, 605, 649, 722, 757, TP015, WP018, TP020, TP027, RP033, MP084, TP159, MP163, MP167, MP172, MP207, MP214, MP222, TP228, WP252  
 ExxonMobil Biomedical Sciences 335 RP138, RP141, RP220  
**F** Fairleigh Dickinson University RP237  
 Fayetteville State University 156  
 Federal Institute of Education, Science and Technology of Rio Grande do Sul, Porto Alegre Campus RP010, MP187, MP213  
 Federal University of Bahia 712, RP113  
 Federal University of Rio Grande MP040, MP187, MP213, WP207, WP219, RP010  
 Federal University of Santa Maria MP040  
 Federal University of Sao Paulo RP201  
 FERA 708  
 Finnish Environment Institute TP064, WP067, RP213  
 First Institute of Oceanography, State Oceanic Administration 699, 700, RP071  
 Fish & Wildlife Research Institute TP105  
 Fisheries and Oceans Canada 97, 195, 196, 197, 200, 202, 227, 240, 255, 340, 404, MP008, MP075, TP054, TP056, TP066, WP082, TP118, TP119  
 Five Winds International RP117  
 Flemish Institute for Technological Research NV ("VITO") 126  
 Florida A&M University WP242  
 Florida Fish and Wildlife Conservation Commission 752, TP196, WP283  
 Florida Gulf Coast University WP283  
 Florida International University 395, 410, 704, TP022, TP027, WP286  
 Florida State University 112  
 Foundation Merieux TP063  
 Food and Environment Research Agency MP195  
 Forschungszentrum Jülich GmbH 334  
 Fort Environmental Laboratories 420, WP052, MP142, MP162  
 Franch Agency for Food, Environmental and Occupational Health Safety 10  
 Fraunhofer IME 334, WP083, WP088, TP088, TP091, RP135  
 French National Institute for Agricultural Research 10  
 Freshwater Institute 442, MP033, TP144, RP253  
 Furman University RP189  
**G** GAIAC 333  
 Gamberg Consulting TP087  
 GEI Consultants 125, 181, 184, 275, 276, 662, 674, RP108, WP128  
 Geochemistry and Geodynamics Research Center (GEOTOP-UQAM) MP016  
 Georgia Aquarium 235  
 Georgia Gwinnett College 533  
 Georgia Southern University WP267  
 Georgia-Pacific Corporation MP043, RP212  
 Geosyntec Consultants MP202  
 German Aerospace Center DLR 453  
 German Federal Environment Agency (UBA) 430  
 German Federal Institute for Hydrology 333  
 Germano & Associates, Inc. 397  
 Ghent University 401, 636  
 Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology 15, WP140  
 GKSS Research Centre 65, MP018, TP040, TP041  
 GlaxoSmithKline 129, WP154, WP155, TP169  
 GLEC Subcontractor WP118  
 Golder Associates 403, 607, MP168, RP101, RP122, RP137  
 Gradient 430, 438, 526, 644, 648, 690, RP003, RP035, TP096, TP097, MP086, MP197, MP207, RP221, TP234, WP239  
 Graduate School of the Chinese Academy of Sciences MP099, WP189  
 Graduate University for Advanced Studies RP251  
 Grand Manan Whale and Seabird Research Station WP110  
 Great Lakes Environmental Center 167, 257, 338, 565, MP014, MP015, MP016, WP118, WP272  
 Great Lakes WATER Institute, University of Wisconsin-Milwaukee 39, 379, MP148  
 Green Science Policy Institute 166  
 Green Seal 687  
 Green Thumb MP087  
 Greenland Institute of Natural Resource TP052  
 GREYC, University of Caen MP001  
 Griffith University TP093

## AFFILIATION INDEX

Groupe de recherche en écologie comportementale et animale (GRECA) MP016  
 Guangzhou Institute of Geochemistry, Chinese Academy of Sciences 260, MP022, MP022, MP094, TP040, TP050, WP189, RP221  
 Guelph University WP144  
 Gustavus Adolphus College MP048  
 Gwangju Institute of Science and Technology 300, MP088, WP152  
**H** Haley & Aldrich, Inc. 601  
 Hamamatsu Photonics K. K. MP181  
 Hangzhou Traffic Administration and Control Centre RP008  
 Hankuk University of Foreign Studies RP169  
 Hanoi National University 457  
 Hanoi University of Science, Vietnam National University 705  
 Hanyang University 136, 139, 315, 458, 469, 520, 522, 561, 563  
 Harbor Branch Oceanographic Institution at Florida Atlantic University 235  
 Harvard School of Public Health 304, TP076, RP235  
 Harvard University 35, 179, 286, 626, 771  
 Hatfield Consultants 182  
 HDR|HydroQual 228, 229, 232, 395, 413, 503, 603, TP022, WP159, RP181, RP216, MP229  
 Health Canada 149, 260, 338, 430, MP108, MP155, WP191, TP229  
 Health Canada Pest Management Regulatory Agency 10  
 Health Physics Society 499  
 Helmholtz Centre for Environmental Research 11, 208, 401, 489, 493, 453, 642, 689, 708, MP018, TP040, TP041, WP072, WP073, WP074  
 Hewlett-Packard 685  
 Hill Air Force Base MP155  
 Hokkaido University, Faculty of Fisheries Sciences TP213  
 Hokkaido University, Graduate School of Veterinary Medicine TP213  
 Hong Kong University of Science and Technology 312, MP034, RP077  
 Hospital for Sick Children MP125  
 House 60, 142 Road MP097  
 Houston Zoo 562  
 HP 685  
 Hydrobiology 415  
 Hydroqual 333, 395 TP022, RP182, MP229  
 Hyogo Prefectural Institute of Public Health and Consumer Sciences Public Health Science Research Center TP270  
**I** IBMB-CSIC 317  
 ICQ – USA 83  
 ICRA 532  
 IDAEA-CSIC 532, TP112  
 Idaho State University RP170  
 IFRA WP016  
 IHR – University of Iowa Civil and Environmental Engineering 552  
 Illinois Natural History Survey RP109  
 ILSI Health and Environmental Sciences Institute 335, 375, TP085  
 Imperial College WP084  
 Independent MP037, MP153  
 Independent Environmental Consultant RP120  
 Indiana University 311, 465, 536, 635, 636, MP013, MP071, TP187, WP208  
 Industrial Economics 427, WP081  
 Industry Task Force II on 2,4-D Research Data MP163  
 INERIS 426  
 Infarmed TP166  
 Infinity Solutions TP020  
 INRA 11, MP150  
 INRB 31, 289, MP027, MP028, TP253  
 INRS 202, 411, 468, WP213, WP217, WP218, WP222, WP231, RP031  
 Institute Maurice-Lamontagne RP006, RP009  
 Institute National de la Recherche Scientifique 409, WP222  
 Institute for Environmental Studies, VU University 19, 23, 93, MP017, RP056, TP057, WP075, TP089  
 Institute of Arctic and Alpine Research 405  
 Institute of Environmental Toxicology 104, 403, WP090, RP101, RP269  
 Institute of Gastroenterology and Haepatology, Cluj-Napoca, Romania RP288  
 Institute of Ocean Sciences 90  
 Instituto Nacional de Ecologia RP154, RP268, RP279  
 Instituto Politecnico Nacional TP179  
 Integral Consulting Inc. 80, 290, 397, 403, WP080

Integrated Systems Toxicology Division 22  
 International Lead Zinc Research Organization, Inc. WP210  
 International Zinc Association 226  
 Interra 294, TP173  
 Intervet Innovation GmbH MP139  
 Intrinsik Environment Sciences Inc. 484, 576, 615, 782, MP169, MP175, MP184, TP207  
 Iowa State University 239, MP232  
 IPIMAR 289, MP027, MP028  
 IQAC 532  
 IRSA-CNR, UOS Brughiero TP076  
 IRTA Carretera de Cabriels 773, RP283  
 ITRI MP017  
**J** Jackson State University 29, 224, 377, 633, RP038, RP090, TP112, TP134  
 Jacksonville State University TP032, WP057  
 Janssen Pharmaceutical Companies of Johnson & Johnson 126  
 Japan, TP141  
 Jean-Michel Cousteau's Ocean Futures Society WP025  
 Jinan University, Institute of Hydrobiology WP189  
 Johns Hopkins University 55, 349, 633, TP195  
 Johnson & Johnson 126, 130, 430  
 Joint Research Centre – European Commission 23  
 Julius Kühn-Institute 10, 547, WP021  
 Justus Liebig University Giessen, Institute of Soil Science and Soil Conservation TP088  
**K** Kansas State University WP253  
 Kaohsiung Medical University RP175  
 Karlsruhe Institute of Technology 333  
 Karolinska Institute MP003  
 Kenyon College WP110  
 Kern Statistical Services, Inc. 485, 514, WP114, MP216  
 Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, Nankai University 368, WP008  
 Khon Kaen University WP188  
 King County Department of Natural Resources 729, MP234  
 KIST Europe 678, RP084  
 Konkuk University 579, WP124, WP170, WP171, WP172, RP203  
 Korea Advanced Institute of Science and Technology WP138  
 Korea Atomic Energy Research Institute WP152  
 Korea Institute of Science and Technology WP159, WP160  
 Korea Institute of Toxicology WP138, RP246, RP260  
 Korea Maritime University WP138  
 Korea Ocean Research and Development Institute 107, 561, RP004, RP034, TP042, TP047, TP151, MP210, TP269  
 Korea Research Institute of Bioscience and Biotechnology RP260  
 Korea Research Institute of Chemical Technology TP126, WP141  
 Korea University 105, RP059, RP063, TP151, WP157, RP169, RP176, MP210, RP246, WP280, WP284  
 Kumamoto Prefectural University MP188  
 Kumamoto University TP051, TP061, TP141, RP184, WP245  
 Kumamoto University, Center for Marine Environment Studies WP245  
 Kunsan National University TP183  
 Kyoto University WP147  
 Kyung Hee University 100, 572, MP124  
**L** Laboratoire de Biotechnologie de l'Environnement, INRA TP073  
 Laboratorio de Ictiofisiología y Acuicultura, IIB-INTECH (CONICET-UNSAM) RP263  
 Laboratorio de Transformación de Residuos, IMYZA, INTA, RP263  
 Lake Biwa Environmental Research Institute WP211  
 Lancaster University 65, 68, 373  
 Lancaster University, Institute of Environmental and Natural Sciences TP146  
 Lancaster University, LEC 537  
 Lasallian Institute for Development and Educational Research De La Salle University MP124  
 Lawrence Berkeley National Laboratory 76, 312, 776, WP160  
 Lawrence University RP022  
 LDEO, Columbia University 351  
 Leibniz Institute of Plant Biochemistry 453, WP073  
 Leopold Ecotox Services 116  
 Leuphana University Lüneburg 9, 586, RP056  
 Level Science Inc. 573, WP032  
 Liege University MP150  
 Lindum Ressurs og Gjenvinning AS 760  
 Linköping University RP043

Linnaeus University, School of Natural Sciences MP003  
 Los Alamos National Laboratory 4  
 Louisiana State University 114, 135, 360, 391, 392.5, WP093, WP227  
 Loyola University Chicago 410, 704, WP286  
**M** M-I SWACO RP163  
 MacDonald Environmental Sciences Ltd. 82, 228, 229, MP241  
 Maine Department of Inland Fisheries and Wildlife WP255  
 Manhattan College 230, 597, 599, 602  
 Marine Biological Laboratory 466, 634  
 Marine Research Institute (CSIC) TP253  
 Massachusetts Department of Conservation and Recreation WP128  
 Massachusetts Department of Environmental Protection 288, RP074  
 Massachusetts Institute of Technology 64, 277, 282, 450, 696  
 Massachusetts Water Resources Authority 589, 591, 593, 595, 693, 695  
 Maxxam Analytics TP082  
 McGill University 320, 324, MP143  
 McMaster University 406, 506, 507, 603, MP040, MP046, MP047, MP049, WP195, WP207, WP210, RP219, WP219, WP225  
 Medical College of Georgia 494  
 Medical University of South Carolina 235, TP075, TP230  
 Meijo University TP148  
 Merck & Co., Inc. 129, TP165, TP169  
 Merck KGaA 129  
 MESL MP238, MP240  
 MESOCOSM GmbH RP135  
 Metro Water Services RP198  
 Metropolitan Water Reclamation District of Greater Chicago RP011  
 Miami University WP136, RP156  
 Michigan Department of Environmental Quality, Waste and Hazardous Materials Division RP062  
 Michigan Department of Natural Resources 379, TP013  
 Michigan State University 11, 27, 117, 378, 379, 381, 471, 514, 568, 719 TP013, MP035, TP075, TP085, WP114, WP157, MP163, MP180, RP181, RP187, MP201, WP253, RP254, WP258  
 Michigan State University, Center for Water Sciences WP099  
 Michigan State University, Wildlife Toxicology Laboratory MP123  
 Michigan Technological University 534  
 Middle Tennessee State University 414, WP047, MP082, RP191, RP198, RP207  
 Milwaukee Metropolitan Sewer District 436  
 Mining Association of BC MP037  
 Ministry of Agriculture 9, 12, MP062, MP064, MP065  
 Ministry of Natural Resources 227  
 Ministry of Water Resources and Meteorology TP063  
 MiPlaza, Philips Research Europe and VU University, Institute for Environmental Studies MP017  
 Mississippi Department of Environmental Quality 396  
 Mississippi Department of Marine Resources 396  
 Mississippi State University 396, 542, MP105, RP185  
 Missouri Department of Conservation WP107, WP115, WP117  
 Missouri State University 350  
 Missouri University of Science and Technology TP069  
 MIT 64, 428, 500, 598, 734, 758  
 Minnesota Dept of Health 367, TP067  
 MOE Key Laboratory of Pollution Processes and Environmental Criteria, Nankai University 368, WP008  
 Moffatt & Nichol 503  
 Monsanto 11, 27, 123, MP201, WP275  
 Montana State University RP170  
 Montani Run, LLC 103, 643, WP015  
 Morehead State University RP278  
 Mote Marine Laboratory 525, TP195  
 Mount Allison University MP047  
 Mount Desert Island Biological Laboratory 222, MP071  
 Murray State University TP039  
 Muséum National d'Histoire Naturelle 202  
 Mutch Associates 230, 597  
 Mystic Aquarium 235  
**N** Nanjing University 677, 680, TP010, TP011, TP012, MP116, WP157  
 Nankai University 368, TP055, WP008  
 NanoSafe, Inc WP166  
 Nanototech Inc. RP176  
 National Aquarium Baltimore 55, TP195

National Council for Air and Stream Improvement RP166  
 National Environmental Research 180, 652, TP052  
 National Health Research Institutes MP159  
 National Hydrology Research Center MP031  
 National Institute for Environmental Studies 22, 138, 175, 254, 256, 298, 301, RP023, TP061, TP141, WP150, MP154, MP181, RP251  
 National Institute of Advanced Industrial Science and Technology WP087  
 National Institute of Biomedical Innovation TP184, TP185, TP188  
 National Institute of Environmental Health Sciences 133, 402  
 National Institute of Health Sciences WP150, TP262, TP270  
 National Institute of Molecular and Isotopic Technologies, Cluj-Napoca, Romania RP288  
 National Institute of Standards & Technology 225, 326, 763, RP068, WP110, WP167, TP075, TP237  
 National Measurement Institute 273  
 National Museum of Marine Biology and Aquarium  
 National Museum of Nature and Science, Tokyo WP026  
 National Oceanic and Atmospheric Administration (NOAA) 52, 144, 168, 391, 393, 394, 404, 427, 467, 770, WP081  
 NOAA, National Marine Fisheries Service 235, 416, 562, WP049, RP021  
 NOAA, National Ocean Service 144, 235, 394, RP232  
 NOAA, Office of Response & Restoration 485  
 National Park Service 441, WP255, WP283  
 National Physical Laboratory 267  
 National Research Council 618, RP100, WP265, WP266, WP268  
 National Research Council, Biotechnology Research Institute 320, 324, 386  
 National Taiwan University TP125  
 National Toxicology Program 133  
 National University of Singapore 158, 264, 356, TP063, TP212  
 National Veterinary Research & Quarantine Service MP103, MP115  
 National Water Research Institute 474, 665, WP030, RP045  
 National Yang Ming University MP159  
 National Institute of Water & Atmospheric Resources 404  
 Natural Resources Canada WP038, WP039, WP040, WP041, RP070, RP071, WP205  
 Natural Resources Canada, Geological Survey of Canada RP122  
 Naturhistoriska Riksmuseet TP077  
 Nautilus Environmental 730, MP037, TP129, RP236  
 Naval Facilities Engineering Command – NW MP110  
 NCDWQ RP178  
 Neff & Associates LLC 605, 607  
 NEMS/MEMS LLC 736  
 NeoEnBiz Co. TP151  
 Neptune and Company Inc. 293, 296  
 NERC Biomolecular Analysis Facility – Birmingham Node 225  
 NERC Centre for Ecology and Hydrology 323  
 New Brunswick Power Corporation 2  
 New England Aquarium WP134  
 New England Interstate Water Pollution Control Commission MP029  
 New Fields Environmental 73, RP002  
 New Mexico State University 4, 350  
 New York State Department of Health WP007, WP011, TP057, RP062, MP087, MP113, TP140, TP141, MP154  
 New York University School of Medicine 52, 466, 467  
 Newcastle University 92  
 NewFields 73, 608, MP189  
 Newfields Northwest 608  
 Nicholls State University RP224, RP240  
 NIFES 94, 262, WP140  
 Nijalingappa College 457  
 NIST 326, TP075  
 NIVA 262, 329, RP094  
 Nordic Institute of Product Sustainability, Environmental Chemistry and Toxicology TP057, MP230  
 Normandeau Associates 594  
 North Carolina State University 40, 148, 213, 505, 508, 679, MP041, MP051, MP137, RP234, RP294  
 North Dakota State University RP080  
 North South Consulting 33  
 Northeast States for Coordinated Air Use Management MP029  
 Northeastern University 319, 621, 625, 626, TP135, WP148  
 Northland College 727  
 Norwegian Geotechnical Institute 86, 429, 760

Norwegian Institute for Agricultural and Environmental Research, Soil and Environment Division 760  
 Norwegian Institute for Water Research 86, 262, 401, 534, MP170  
 Norwegian Polar Institute 66, WP075  
 Norwegian School of Veterinary Science WP075  
 Norwegian University of Science and Technology 56, 767, 768, WP075, WP140  
 Norwegian University of Science and Technology, Department of Biology 609, RP094  
 Norweigen Geotechnical Institute 91  
**O** Oak Ridge Institute for Science and Education MP236, WP254  
 Oak Ridge National Laboratory RP075  
 OARS 432  
 Obafemi Awolowo University WP262  
 Oceanic Institute WP060  
 OECD 28, 329  
 Office of Environmental Health Hazard Assessment WP025  
 Office of the National Steering Committee 33 182  
 Office of the State Chemist – Mississippi 396  
 Ohio University RP232  
 Ohio State University 37, 354, 386, 545, TP104, TP238, WP238, TP267, WP238, WP276, RP146  
 Ohio State University, Department of Chemistry RP049  
 Ohio State University, Department of Evolution, Ecology and Organismal Biology RP049  
 Ohio State University, School of Earth Sciences RP049  
 Okazaki Institute for Integrative Bioscience MP078  
 Oklahoma State University 248, 249, 384, 385, 538, WP048, TP177, TP178, RP195, TP197, WP269  
 Ontario Ministry of the Environment 244, 442, 484, 555, 556, 557, 672, MP008, TP054, MP055, RP102, TP120, TP144, WP179, WP180, TP207, WP260  
 Oregon Episcopal School TP205  
 Oregon Health & Science University 679  
 Oregon State University 501, 780, WP002, WP068, TP107, WP119, RP142, TP246, TP248  
 ORNL / UT-Battelle MP177  
 Osaka University TP184, TP185, TP188, MP228, MP233, MP235  
 Otsuka Pharmaceutical Co. Ltd. TP162  
 Oxford University 286, 323  
**P** Pacific Biodiesel Technologies WP060  
 Pacific EcoRisk 614  
 Pacific Northwest National Laboratory WP068, MP110, RP038  
 Parametrix, Inc. TP022, TP027  
 Parsons 549  
 Patrick Center for Environmental Research, Academy of Natural Sciences TP046  
 Patuxent Wildlife Research Center WP181, WP182, WP187, WP261, WP270  
 Paulista State University RP201  
 PE International RP117  
 Peking University RP063  
 People for the Ethical Treatment of Animals 121  
 Peregrine Fund 753  
 Pest Management Regulatory Agency MP063  
 Pfizer Global Research and Development 124, 129, 130, WP146, RP188  
 Philadelphia University 83, TP046, MP156  
 Physicians Committee for Responsible Medicine 121  
 Polar Research Institute of China TP040  
 Polaris Applied Sciences, Inc.  
 Polish Academy of Sciences RP126  
 Politecnico di Milano 92  
 Polo APTA Vale do Paraiba MP091  
 Pontificia Universidade Católica do Rio de Janeiro TP048, RP073, RP076  
 POPs Environmental Consulting 720  
 Pratt School of Engineering 145, 153, 218, RP013, MP024, MP111  
 Prefectural University of Kumamoto MP188  
 Prince Edward Island Department of the Environment 484  
 Principal Consultant 95, 398, 403, WP192  
 Prof. Dr. Assen Zlatarov University 208  
 Proteomics Core Facility, Institute for Genome Science & Policy, Departments of Cell Biology, Medicine, and Neurobiology, Duke University Medical Center 218  
 Public Health Institute 455  
 Public Works and Government Services Canada 189  
 Puget Sound Naval Shipyard & IMF MP110  
 Purdue University 18, 524, 714, 770, WP139, WP165, TP187  
 Pusan National University 440, 458, TP009, WP248

**Q** Quantum Management Group, Inc. TP169  
 Queen's University 169, 195, 196, 197, 198, 199, 200, 448, 580, 628, MP015, MP070, MP132, WP077, RP167  
 Queens College, CUNY 351  
**R** Radboud University RP116  
 Raytheon Company 261  
 Reading University 89  
 Rensselaer Polytechnic Institute 544  
 Research Institute for Chromatography RP066  
 Research Institute for Fragrance Materials 203, 528, WP016  
 Research Planning Inc. 391, 427, TP031, WP081  
 Rhode Island Department of Environmental Management RP239  
 Rice University TP098  
 Richardson College for the Environment WP004  
 Rifcon GmbH WP089  
 Rio Tinto 232, 413, 673, RP140  
 RIVM 667  
 Rochester Institute of Technology WP105  
 Roger Williams University 50, WP134, WP135, RP226, RP241, RP280  
 Roosevelt University TP190, TP209  
 Rosenstiel School of Marine & Atmospheric Sciences 463, 466  
 Roskilde University 203, 652, WP085, WP086  
 Rothamsted Research 493  
 Rowan University 247  
 Royal Military College of Canada 702, MP132, WP193, WP196  
 Royal Roads University RP122  
 Rutgers, The State University of New Jersey 360, 535, 718, TP030, TP111, TP113, TP131, RP165, WP201  
 RWTH Aachen University 333, 334, 489, 710, 711, 713, WP088  
**S** Sabic Innovative Plastics TP001, RP193  
 Saitama City Institute of Health Science & Research WP147  
 San Diego State University 655, RP068, RP155, WP204  
 San Francisco Estuary Institute 166, 669, RP068, MP192, TP216  
 Sangmyung University 136, 469, 563  
 Sanitation Districts of Los Angeles County RP153  
 Santa Cruz University RP113  
 Sanyo Techno Marine, Inc. WP220  
 Saskatchewan Research Council 577  
 Sasol Technology MP196  
 Savannah River National Laboratory 494  
 Savannah State University WP023, TP039, TP146  
 SC Johnson & Son 335, MP142  
 School of Environmental and Life Sciences TP197  
 School of Environmental Health 173, TP121  
 Science and Technology Branch 167, 257, 338, 565, MP014, MP015, MP016, WP272  
 Scottish Universities Environmental Research Centre (SUERC) RP012  
 Section of Marine Ecology and Biotechnology, Division of Life Science MP034  
 Seoul National University 430, 639, WP046, RP078, TP101, WP138, MP145, TP151, WP157, TP199, MP210, RP218, WP235, RP246, RP260, RP290  
 SETAC North America 109, 110, RP282  
 Seton Hall University 360, RP159  
 Shanghai Jiao Tong University TP011, TP012, MP116  
 Shaw Environmental & Infrastructure TP006  
 Shaw Group 183  
 Shell Canada 470, WP042  
 Shell Health 335, 470, 578, WP042  
 Shell Oil Company, Shell Health RP138, RP141, RP220  
 Shimane University, Faculty of Medicine 707  
 Shinshu University 707  
 Silent Spring Institute 74, 304, WP013  
 Simon Fraser University 90, 93, 204, 240, 265, 340, 498, RP037, TP049, TP066, TP080, TP081, TP089, TP119, TP121, TP127, MP144, MP200, MP247, WP272  
 SINTEF 216, 313, TP019, TP021  
 SINTEF Fisheries and Aquaculture 767, 768, RP094, WP140  
 SINTEF Materials and Chemistry 609, 767, RP094, WP140  
 Skokomish Tribe 237  
 Smithers Viscient RP132, MP136, RP139, WP155, MP164, MP165, TP167, RP193, TP256, WP258  
 Smithsonian National Zoological Park 70, 550  
 SNC-Lavalin Environment 189  
 Soil Protection Technical Committee (TCB) 746  
 Sole Trader 9



## AFFILIATION INDEX

Solutia Inc. 79, 84  
Soonchunhyang University 639, TP151  
South Carolina Dept of Natural Resources 763  
South China Normal University 260  
South China Normal University, College of Life Science,  
Key Laboratory of Ecology and Environmental Science in  
Guangdong Higher Education 369, TP139  
Southern California Coastal Water Research Project 451, 669,  
676, RP068, MP204  
Southern Illinois University 88, 403, 607, WP065, WP066,  
MP095, MP131, RP200, MP245, TP179, TP259  
Southern Illinois University at Edwardsville WP139, WP149  
Southern Illinois University Carbondale 88, WP065, WP066,  
MP095, MP131, TP177, RP179, RP195, RP221, TP259  
Space and Naval Warfare Systems Center MP110  
Spanish Council for Scientific Research TP141  
SPAWAR Systems Center Pacific 384, 730, RP155, WP204  
SpecPro Inc. 377, 633, 681, WP263  
Springborn Smithers Laboratories MP147, MP164  
SRC Inc. 171, 641, MP217, TP240  
St Cloud State University 221, 357, RP011  
St Johns River Water Management District 751, 752  
St Paul's Hospital TP121  
Stanford University 85, 759  
Stantec MP098, MP175  
State of Alaska 495  
State of California, Department of Public Health TP136  
State University of Campinas 154  
State University of New York at Albany WP011  
State University of New York College of Environmental  
Science and Forestry 61, TP200, TP233  
State University of New Fredonia 164, 165, MP054, MP058  
State University of New York Oswego 164, 165, MP054,  
MP058  
State University of New York Upstate Medical University  
MP242  
Stearns & Wheeler GHD RP024  
Stockholm University 63, 205, 312, 373, 399, 760, TP057,  
TP218, MP230  
Stone Environmental Inc. MP169, MP184, MP186  
Stony Brook University 237, 657, 694, RP040, RP123  
Stratus Consulting 299, 393, 750, WP119  
Stroud Water Research Center 508, MP041, TP252  
Student Contractor to the USEPA 72, 272, 301, TP203,  
TP204  
Student Services Contractor to USEPA 47, MP048  
Summit Toxicology 722  
Sustainable Sciences LLC TP169  
Sustainable Intelligence LLC RP118  
Swedish University of Agricultural Sciences 68, 255, 442, 443,  
TP144  
Swiss Federal Institute of Aquatic Science and Technology 375  
Swiss Federal Institute of Technology Zürich 773  
Swiss Federal Laboratories for Materials Science and  
Technology TP087  
Syngenta 10, 491, 493, 724, 725, MP178, MP183, MP186,  
WP084, WP086, WP091, WP092, RP133, RP157, RP252  
Syracuse Research Corporation 208  
Syracuse University 38, WP191, TP200  
Tallinn University of Technology TP070  
TAMAR-ICMBio WP050  
Tartu University TP070  
TCB 746  
TechLaw, Inc. WP122  
Technical University of Denmark 733, 774, WP200, WP233  
Technische Universität Berlin WP021  
Technischen Universität Hamburg-Harburg RP036  
Technology Center for Marine Survey MP124  
Temple University 591  
Tennessee State University RP016, RP019, MP102, MP106,  
MP129, MP136  
Tennessee Valley Authority 96, MP177  
TestAmerica 58  
Tetra Tech Inc. 637, 664, RP106, TP225, TP226, RP262  
Texas A&M University 512, WP095, WP139, RP198  
Texas A&M University, Corpus Christi 696  
Texas Christian University 32, MP092, TP230, RP273  
Texas State University RP216  
Texas State University – San Marcos RP273  
Texas Tech University 187, 416, 538, 578, TP023, WP045,  
WP048, WP058, WP059, TP060, TP069, WP106, MP141,  
RP168, TP182, TP189, RP200, RP206, RP210, RP211,  
RP228, RP242  
Texas Tech University / TIEHH 538, 562, 578, MP141,  
TP023, TP060, TP069, TP182, TP186, TP189, WP045,  
WP049, WP059, WP093, RP271  
Texas Tech University Health Sciences Center Department of  
Cell Physiology and Biophysics WP049  
The Citadel 692  
The College of Staten Island  
The College of William and Mary TP193, WP277  
The College of Wooster 357  
The George Washington University 778  
The Hamner Institutes for Health Sciences 22  
The Hawaii Land Restoration Institute WP060  
The Hong Kong Polytechnic University 604  
The Keck Institute 633  
The Loper Group 80, 84  
The Marine Mammal Center RP068  
The McConnell Group – USEPA 298, MP041, TP187, WP137  
The Pennsylvania State University 11, TP249  
The Procter & Gamble Company 103, 329, 335, 375, 527,  
643, TP085, WP015, WP019, WP125, RP139, RP156  
The Science Collaborative MP193  
The Shaw Group 183  
The Third Institute of Oceanography, State Oceanic  
Administration WP099  
The University of Findlay WP100  
The University of Iowa 370  
The University of La Verne 234 WP104, TP116, TP117  
The University of Shiga Prefecture RP096  
The University of Tokyo RP023  
Thermo Fisher Scientific MP019, TP078, TP079, RP196  
Tochigi Prefectural Museum MP007  
Tokyo Institute of Technology WP209  
Tokyo Metropolitan Institute of Public Health 256, WP150,  
TP262, TP270  
Tokyo National College of Technology MP191  
Tongji University MP120, RP265  
Tottori University 705, MP007, WP211  
Towson University RP022, MP023, RP025, RP125, RP128,  
RP129, WP237  
toXcel LLC WP259  
Toxic Action Network Central Asia MP199  
Toxicology Centre WP030  
Toyo University WP162  
Transport Canada 189  
Trent University 36, 45, 204, 244, 263, 309, 435, 627, 656,  
WP019, MP030, MP033, TP223, TP231, WP232, TP236,  
TP244  
TRIUM Environmental Inc. 56  
Troy University RP266  
Tufts University 646, 735, RP001  
Tulane University 779  
TVA Kingston Ash Recovery Project 96, MP177  
UAM Iztapalapa MP053, RP154, MP173, MP190,  
RP268, RP279  
UBA, Umweltbundesamt TP166  
UBA, German Federal Environmental Agency 329  
UF/IFAS-IRREC 726  
UFSC 132  
UFZ, Helmholtz Center for Environmental Research 375,  
401, 453, WP072, WP073, WP074  
UGent 636  
Ulsan National Institute for Science and Technology 458  
Umea University 68  
UNC, EPA TP053  
Unesp 151, 155  
UNICAMP 148, 151, 154, 155, WP076  
Unidad de Ciencias del Agua-CICY WP135, RP280  
Unilever 129, 399, 433, 530, WP014, RP029  
Union College TP235, TP265, WP273  
Unit of Work Environmental Toxicology MP003  
United Nations Environment Program (UNEP) RP282  
Universidad Autonoma de Baja California TP264  
Universidad Autonoma Metropolitana MP053, RP154,  
MP173, MP190, RP268, RP279  
Universidad de Castilla, La Mancha 415  
Universidad de Chile RP197  
Universidad de Concepcion 505  
Universidad de la República  
Universidad de Sonora MP089, MP090  
Universidad Nacional Autónoma de México 50, MP190,  
WP135, RP226, RP241, RP280  
Universidade de Aveiro 323, 415  
Universidade de São Paulo MP091, WP199  
Universidade do Porto 415  
Universidade Estadual de Maringá 155, MP092  
Universidade Estadual de Campinas 152, WP076, RP201  
Universidade Estadual do Norte Fluminense MP157, RP072  
Universidade Federal de Parana RP009  
Universidade Federal de Santa Catarina 132, MP076  
Universidade Federal de São Paulo WP076  
Universidade Federal do Espírito Santo WP203  
Universidade Federal do Rio de Janeiro RP072, RP073,  
RP076, MP157  
Universidade Federal do Rio Grande 53, 408, 507, MP076,  
MP077, TP043, TP045, TP045, TP180, WP195, WP202,  
WP203, WP234  
Universita Cattolica 500  
Università degli Studi di Firenze 537, RP043  
Università degli Studi di Napoli Federico II 415  
Université Bordeaux 1 202  
Université de Montréal WP278  
Université de Toulouse 44  
Université du Québec 32, TP194, TP230  
Université du Québec à Montréal MP009  
Université du Québec à Rimouski 200, MP075, RP006,  
RP009  
Université Laval 202, 468, WP082  
Universités Bordeaux 1 et 2 TP112  
Universiti Kebangsaan Malaysia WP240  
University at Buffalo  
University at Buffalo SUNY WP049  
University Center on Svalbard 66  
University Centre in Svalbard (UNIS)  
University Hospital of Antwerp 178  
University of Agricultural Sciences and Veterinary Medicine,  
Cluj-Napoca, Romania RP288  
University of Agriculture WP006  
University of Akdeniz TP073  
University of Alabama 134, 466, 634  
University of Alabama at Birmingham 532, 629  
University of Alaska Anchorage 383  
University of Alaska Fairbanks 608, 610  
University of Alberta 37, 173, 174, 241, 255, 341, 471, 472,  
568, 571, 572, WP029, WP031, WP037, RP039, RP042,  
RP141, WP043, TP114, TP121, RP229  
University of Amsterdam TP057  
University of Antwerp 176, 178, 374, RP065, MP126, TP142,  
TP143  
University of Arizona 4, TP003  
University of Arkansas RP085, RP185  
University of Arkansas, Pine Bluff 763  
University of Aveiro 323, 415  
University of Basel 341, 632  
University of Bern TP085  
University of Birmingham WP001, MP126, MP127, RP038  
University of British Columbia 173, 220, RP093, TP058,  
TP118, TP121  
University of California 75, 88, 141, 312, 322, 402, 650, 764,  
776, WP003, WP024, MP044, TP072, TP164, WP178,  
TP254  
University of California, Berkeley 312, 358, 402, 418, 451,  
754, 776, TP128, WP101, WP102, WP159, WP160, TP187,  
RP038  
University of California, Davis 71, 75, 318, 400, 447, 650,  
764, MP159, TP164, TP254, WP003, WP024, WP178,  
RP107, RP174, RP214  
University of California, Riverside 400, MP107, TP059,  
TP072, TP059, RP026, RP027, RP214, RP274  
University of California, Santa Barbara 142, 489, TP132,  
TP183, WP250, RP256  
University of California and Lawrence Berkeley National  
Laboratory 312, 402, 776  
University of California, San Diego 730  
University of California South Coast Research and Extension  
Center RP026, RP027  
University of Cartagena TP148  
University of Cincinnati 22, 254, 456, 640  
University of Colorado WP274  
University of Colorado at Boulder 405  
University of Colorado School of Medicine WP267  
University of Connecticut 35, 512, 728, RP020, TP025,  
WP053, TP094  
University of Copenhagen WP200  
University of Crete 272  
University of Delaware 351, 387, 388, 599, 602, MP002,  
TP084, TP099, TP100, WP158, TP252  
University of Dschang RP233  
University of East Anglia 762, TP152

- University of Eastern Finland 59, 326, 606, TP064, WP067, MP100, TP124, RP213, TP218
- University of Eastern Finland, Joensuu Campus 606, 660, MP100
- University of Exeter RP012, MP081, WP084
- University of Findlay WP100
- University of Florida 11, 20, 221, 247, 314, 317, 342, 343, 352, 382, 419, 425, 640, 723, 727, 732, 752 RP013, RP015, MP043, RP046, WP078, MP078, WP084, RP086, WP102, TP123, TP124, RP142, WP165, WP174, RP177, RP212, RP295
- University of Florida, IFAS 726, TP068
- University of Florida, Gulf Coast Research & Education Center 723, RP015
- University of Georgia 21, 144, 385, 701, WP097, RP105, WP112, RP162, TP209, WP267, WP274
- University of Georgia, College of Public Health, Department of Environmental Health Science MP113, TP140
- University of Gothenburg 48, MP198
- University of Guelph 11, 437, 569, 588, 615, 619 MP012, WP033, WP036, WP044, TP087, RP102, MP140, WP144, WP145, RP157, WP205, TP207
- University of Health Science TP063
- University of Heidelberg 329
- University of Helsinki 59
- University of Houston 721
- University of Houston Clear Lake TP016, TP018, RP134
- University of Ibadan MP151, MP152
- University of Illinois at Urbana-Champaign 6, RP109
- University of Ilorin RP243
- University of Insubria 208, 583, 683
- University of Iowa 170, 370, 539, 541, 551, 552, 756, TP147
- University of Joensuu 606
- University of Johannesburg MP080
- University of Jyväskylä TP062, TP064, RP213
- University of Kentucky 327, RP183, TP190, TP202, TP209, RP278
- University of Koblenz-Landau 678, RP084
- University of Kumamoto TP261
- University of La Verne 234 WP104, TP116, TP117
- University of Lancaster MP097
- University of Las Vegas 235
- University of Lethbridge 519, TP093, RP215, WP256, WP282
- University of Louisiana RP255
- University of Louisiana at Lafayette RP173, RP247, RP255
- University of Louisiana at Monroe 222
- University of Louisville 214, 363, TP015
- University of Maine 3, 697, TP115, RP115, RP192, RP284, RP285
- University of Malta 76
- University of Manchester RP124
- University of Manitoba 67, 227, 283, 588, WP004, TP008, WP144, RP157, MP158, TP168, RP202, RP238
- University of Mansoura
- University of Maryland 13, 17, 111, 303, 519, RP025, MP096, WP186, WP256, WP282
- University of Maryland Baltimore County 192, 761, MP101, MP174
- University of Maryland, Wye Research and Education Center 17
- University of Massachusetts 734
- University of Massachusetts Amherst 343, 432
- University of Massachusetts Boston 425, 559, 596, 693, 697, 699, 700, RP051, RP052, RP074, WP129, WP132, WP134, WP176, TP187
- University of Massachusetts Dartmouth 201, MP069
- University of Massey MP126
- University of Medicine and Dentistry, New Jersey TP111
- University of Medicine and Pharmacy Cluj-Napoca, Romania RP054, RP288
- University of Messina 565
- University of Miami RP161
- University of Miami, RSMAS 232, 673, WP131, WP214, WP229
- University of Michigan 142, 336, 339, 378, 379, 381, 407, 560, 773, 777, RP083, TP105, TP108, WP184, TP196, RP235, WP266, WP018, WP198, RP235
- University of Michigan-Dearborn WP098
- University of Minnesota 306, 445, 653 TP054, MP149, RP222
- University of Minnesota Duluth TP095
- University of Mississippi TP026, MP073, WP163, TP268
- University of Missouri 736, WP115
- University of Montana 16
- University of Natural Resources and Applied Life Sciences 89
- University of Nebraska 9, 24, 159, 203, 305, 770, WP085, WP086, WP177
- University of Nebraska Medical Center 24, 137, RP013
- University of Nevada WP020
- University of New Brunswick 219, 342, 380, 637, 665, 727, MP045, MP047, MP072, MP209, WP082, RP098, TP129, TP130, MP209, RP259, RP295
- University of New Brunswick Saint John TP127
- University of New Hampshire 362, 698
- University of New Orleans TP005
- University of North Carolina at Chapel Hill 348, 464, TP163
- University of North Carolina at Charlotte 40, WP133, RP208, RP294
- University of North Carolina Wilmington WP110
- University of North Dakota 91, WP069, TP208
- University of North Texas 32, 738, MP092, WP146, WP151, RP161, WP168, MP170, RP188, TP211, TP230, TP271, RP277, RP281
- University of Notre Dame 635
- University of Oklahoma 215
- University of Ontario Institute of Technology 331
- University of Oslo 86
- University of Otago TP238
- University of Ottawa 27, 263, 338, 518, 719 RP070, RP071, TP247
- University of Oxford 285, 286
- University of Pennsylvania School of Medicine TP241, RP007
- University of Pittsburgh WP133
- University of Plymouth 43, 509, 570, WP031, WP098, WP175
- University of Porto, ICBA and CHIMAR MP052, TP106
- University of Prince Edward Island 567, MP209, WP028, WP036, RP091, WP215
- University of Puerto Rico and Universidad Metropolitana Cupey Campus 783
- University of Quebec INRS RP281
- University of Reading 31, RP124
- University of Regina 255 RP045
- University of Rhode Island 69, 106, 255, 553, 590, 654, 697, 755, 758
- University of Rouen RP233
- University of San Diego WP204
- University of Saskatchewan 27, 44, 67, 105, 470, 471, 568, 680, 719, 748 RP012, MP026, MP031, WP035, WP040, WP041, RP063, RP070, RP071, MP098, TP151, WP157, RP181, RP186, RP187, MP201, RP202, RP204, RP205, WP221, RP248, RP254, WP262, WP226
- University of Seoul 325, RP078, WP142, WP143, RP290
- University of Shizuoka TP162, TP257
- University of Simon Fraser RP037
- University of South Alabama TP026
- University of South Carolina 145, 375, 732, RP082, RP232
- University of South Carolina Beaufort 343
- University of South Carolina Aiken 327
- University of South Dakota 417
- University of South Florida 247, 404, 419
- University of Southern Denmark 287
- University of Southern Maine 554, RP017
- University of Southern Mississippi RP295
- University of Southern Mississippi, Gulf Coast Research Lab RP261
- University of St. Thomas 221, RP011, TP109, TP110, RP151
- University of Stellenbosch MP196
- University of Stuttgart 773
- University of Sussex 449
- University of Tampa WP062
- University of Tennessee 28, WP098, WP175
- University of Tennessee at Chattanooga TP008, TP168, RP238
- University of Texas 756
- University of Texas Medical Branch, Galveston RP148
- University of Texas at Arlington WP095
- University of Texas at Austin MP231
- University of Texas at El Paso 157, 350, TP002, RP171, TP258, RP225
- University of the Ryukyus 138
- University of Tokushima WP147, RP144
- University of Toronto 252, 253, 310, 324, 442, 555, 556, 557, 600, 627, 766, MP004, MP021, TP054, TP057, RP067, TP120, TP144, MP155, WP179, WP180, WP232, RP244
- University of Toronto Scarborough 204, 208, 399, 442, 642, 689, 766
- University of Umea 68
- University of Victoria 220, MP033
- University of Virginia 38
- University of Warwick 493
- University of Washington 51, 402, RP018, TP059, RP270
- University of Waterloo 37, 195, MP030, WP034, WP044, MP047, WP061, MP125, TP130, MP140, RP209, TP250, TP251
- University of Windsor 207, MP079, TP086, TP090, TP263
- University of Winnipeg WP004
- University of Wisconsin 236, 436, RP152, WP169, WP185, RP293
- University of Wisconsin Milwaukee 39, 147, 381, 436, TP013, WP103, TP105, TP107, MP148, WP176
- University of Wollongong RP217
- University of Wyoming 182, 299
- University of York 402, 430, 433, 708 WP154
- University of Zambia TP213
- University of Zlatarov 208
- University of Zürich-Irchel 632
- Uppsala University, Department of Biochemistry & Organic Chemistry Laboratory MP003
- URS Corporation 95, MP109, WP192
- US Army Corps of Engineers 1, 5, 8, 82, 385, 422, 423, 425, 428, 500, 703, 733, 734, 786, MP238, MP240, WP078, RP086, RP089, WP094, TP102, MP226, WP249, WP254, WP276
- US Army Defense Threat Reduction Agency TP099
- US Army Edgewood Chemical Biological Center 386, TP099, TP104
- US Army Engineer Research and Development Center 1, 29, 152, 188, 224, 359, 377, 384, 404, 422, 423, 425, 428, 429, 500, 633, 703, 733, 734, 736, 786, MP226, MP237, MP239, MP241, TP014, TP097, TP098, TP103, TP112, TP132, TP134, TP201, WP078, WP094, WP159, WP163, WP166, WP249, WP250, WP263, WP271, WP276, RP038, RP081, RP086, RP090, RP250, RP256, RP286, RP287, RP291
- US Army Institute of Public Health 585
- US Army Public Health Command 385, 574, 585, 701, WP112, WP254
- US Department of Agriculture (USDA) 10, 13, 17, 302, MP096, MP223, TP182, TP1829, WP279, RP088
- USDA Agricultural Research Service, National Sedimentation Lab MP223, RP088, RP216
- USDA NCAUR-ARS 710
- USDA Wildlife Services 511
- US Department of Energy (USDOE) 294, TP173
- USDOE, Richland Operations Office 510
- US Environmental Protection Agency (USEPA) 9, 17, 18, 22, 25, 26, 29, 30, 41, 47, 54, 72, 79, 81, 82, 122, 135, 145, 161, 193, 194, 206, 218, 223, 224, 242, 254, 261, 272, 298, 301, 330, 335, 346, 375, 380, 400, 462, 446, 450, 452, 466, 487, 488, 502, 531, 533, 534, 558, 559, 581, 587, 617, 618, 628, 630, 638, 661, 664, 666, 679, 739, 741, 744, 755, 757, 765, 769, 772, MP029, MP041, MP042, MP048, MP093, MP128, MP133, MP138, MP149, MP151, MP152, MP208, MP237, MP238, MP239, MP176, MP240, MP241, TP004, TP005, TP006, TP007, TP031, TP053, TP060, TP071, TP085, TP134, TP138, TP170, TP171, TP187, TP203, TP204, TP206, TP208, TP210, TP227, TP239, TP245, TP260, WP020, WP055, WP079, WP118, WP122, WP129, WP130, WP136, WP137, WP164, WP185, WP194, WP265, WP268, WP281, RP078, RP090, RP097, RP100, RP103, RP104, RP106, RP110, RP151, RP162, RP222, RP239, RP257, RP262, RP266
- USEPA Environmental Response Team 82, 109, 110, MP237, MP238, MP241
- USEPA Mid-Continent Ecology Division 221, TP109, TP110, RP151
- USEPA National Center for Environmental Assessment RP038
- USEPA National Health and Environmental Effects Research Laboratory 106, 145, 261, 446, 452, WP020, TP227
- USEPA National Exposure Research Laboratory 452, 558, 638, 765
- USEPA Office of Chemical Safety and Pollution Prevention, Antimicrobials Division, Design for the Environment Program TP240
- USEPA Office of Pesticide Programs, Office of Research and Development 575, MP171
- USEPA Office of Research and Development, National Risk Management Laboratory 17, 29, 193, 254, 298, 301, 533, 558, TP004, MP041, TP170, TP171, TP187, TP225, TP226, TP260
- USEPA Office of Superfund Remediation and Technology Innovation 553
- US Food and Drug Administration TP033, TP034
- US Fish and Wildlife Service 82, 201, 404, 495, 511, 515, 517, 612, 751, 752, 769, MP069, MP078, MP138, TP164,

## AFFILIATION INDEX

WP111, WP113, WP120, WP181, WP182, WP255, WP286, RP025, RP178, RP199, RP249	Veolia Water North America 436	Wellington Laboratories MP012
US Fish and Wildlife Service, Northern Idaho Field Office 510	Vermont Department of Environmental Conservation MP029	Wesleyan University 582
US Navy 102, MP110	Virginia Institute of Marine Science 17, 355, 443, 592, RP043, WP187	West Texas A&M University WP096, RP276
US Geological Sciences 18	Virginia Tech 131, 701, RP150, WP261, WP270	Western Kentucky University & Mammoth Cave National Park RP019
US Geological Survey (USGS) 38, 70, 82, 98, 153, 193, 228, 229, 243, 246, 305, 344, 345, 346, 357, 366, 382, 400, 405, 421, 432, 439, 441, 444, 445, 504, 510, 513, 515, 550, 663, 668, 698, 752, 769, MP029, MP038, MP039, MP102, MP106, MP136, MP137, MP138, MP237, MP238, MP239, MP240, MP241, TP149, TP156, TP176, WP027, WP054, WP107, WP115, WP116, WP117, WP185, WP223, WP224, WP226, WP230, RP050, RP104, RP143, RP170, RP178, RP199, RP228, RP249, RP272	Visiting Dupont Research Scientist at Duke University 218, RP013	Western Washington University 104, 108, 403, WP090, RP101, RP269
USGS Biological Resources Division 228, 229, 382, 696, RP104, WP107, WP117, TP004, TP123, RP019, RP033, RP143, MP237, MP238, MP241	Vrije Universiteit Amsterdam 19, 93, 323, 489, 631, MP017, TP089	Weston Solutions Inc. 189, 278, 279, 280, 743
USGS Colorado State University 431	<b>W</b> Waco Regional Water Quality Laboratory TP215	Wheatcraft and Associates 645
USGS Colorado Water Science Center 441	Wadsworth Center RP062, TP148	Wildlife International Ltd. 116, TP211
USGS Columbia Environmental Research Center 344, 345, 441, 696, WP248, RP104	Wadsworth Center and Department of Environmental Health Sciences, SUNY at Albany WP011	Wilfrid Laurier University 143, 406, 412, 572, 603, MP045, MP046, MP047, MP073, WP206, WP219, WP228
USGS Georgia Cooperative Fish and Wildlife Research Unit 21	Wadsworth Center New York State Department of Health and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany 368, 372, WP008, MP113, TP140, WP011, RP062	Windward Environmental LLC 185, 226, 232, 478, 673, 742
USGS Great Lakes Science Center TP013	Wadsworth Center, SUNY Albany TP061	Wisconsin Department of Natural Resources 336, WP185
USGS Patuxent Wildlife Research Center WP181, WP182, WP187, WP261, WP270, WP279, RP105, RP162	Wageningen University, RIKILT 266	Wisconsin State Laboratory of Hygiene RP152, RP293
US Research and Development Center WP166, WP271, WP276, RP291	Wake Forest University 40, RP294	Woodard & Curran 295, 297
Utah State University 76, 548, 549, MP155	Washington Closure Hanford 293, 295, 296, 297	Woods Hole Group MP193
Utrecht University WP069	Washington Cooperative Fish and Wildlife Research Unit, University of Washington 51, RP018, RP270	Woods Hole Oceanographic Institution 27, 113, 133, 134, 212, 460, 463, 466, 467, 562, 590, 593, 634, 652, 695, 696, 698, 719, MP076
UVic-Genome BC Proteomics Centre 220	Washington River Protection Solutions 290	Wright State University TP230
<b>V</b> Valdosta State University MP160, WP197, WP216	Washington State Department of Ecology TP174	WSP Environment & Energy MP215
Veolia Environnement Recherche et Innovation SNC RP056	Washington State University 486, 753 RP046, WP064, RP142, MP198	<b>XY</b> Xerces Society for Invertebrate Conservation 10
	Waterborne Environmental Inc. 724, 725, MP183, WP014	Yale University WP053, RP053
	Waters Corporation RP066, TP242, TP266	Yantai Institute of Coastal Zone Research, CAS TP040
	Waters Technologies Ltd. WP008	Yeongsan River Environmental Research Center MP088
	Waterways Experiment Station EP-R 188, 377, RP086, MP226, WP263	Yokohama national university 256
	Weatherford 217, TP035	York University WP206
		<b>Z</b> Zajdlik & Associates Inc. MP098
		Zemo & Associates 543
		Zhejiang University WP099



- 5<sup>th</sup> International Symposium on Selenium-Mercury Interactions** MP157, MP158, MP159, MP160
- A Tribute to Lemna: Ecotoxicology's Greatest Toxicity Test** MP059, MP060, MP061
- Advancements in Environmental Risk Assessment and Management of Pharmaceuticals in the Environment** TP165, TP166, TP167, TP168, TP169
- Advances in Animal Alternatives in Ecotoxicology** 328, 329, 330, 331, 332, 333, 334, 335, TP179, TP180, TP181
- Advances in Assessing (Bio)availability of Organic Pollutants in Sediment/Soil for Site Characterization and Remediation** 85, 86, 87, 88, 89, 90, 91, 92 MP094, MP095, MP096, MP097, MP098, MP099, MP100, MP101, MP102
- Advances in Ecological Modeling for Assessing Ecological Risks and Ecosystem Services** 486, 487, 488, 489, 490, 491, 492, 493, WP078, WP079, WP080, WP081, WP082, WP083, WP084, WP085, WP086, WP087, WP088, WP089, WP090, WP091, WP092, WP093
- Advances in Egg Injection and Embryo Toxicity Studies in Birds and Reptiles** WP181, WP182, WP183, WP184, WP185, WP186, WP187, WP188
- Advances in Environmental Sampling and Analysis** MP103, MP104, MP105, MP106, MP107, MP108, MP109, MP110, MP111, MP112, MP113, MP114, MP115, MP116, MP117, MP118, MP119, MP120, MP121, MP122, MP123
- Advances in Environmental Sampling and Analysis – Part 1** 55, 56, 57, 58, 59, 60, 61, 62
- Advances in Environmental Sampling and Analysis – Part 2** 156, 157, 158, 159, 160, 161, 162, 163
- Advancing the OMICS for Aquatic Ecotoxicology and Ecology** TP123, TP124, TP125, TP126, TP127, TP128, TP129, TP130, TP131, TP132, TP133, TP134, TP135
- Advancing the OMICS for Aquatic Ecotoxicology and Ecology – Part 1** 218, 219, 220, 221, 222, 223, 224, 225
- Advancing the OMICS for Aquatic Ecotoxicology and Ecology – Part 2** 312, 313, 314, 315, 316, 317, 318, 319
- Adverse Outcome Pathway Framework: Moving from Theory to Practice** 375, 376, 377, 378, 379, 380, 381, 382 TP013, TP014
- Air Quality and Nanotoxicology** RP006, RP007, RP008, RP009, RP010
- Amphibians and Reptile Ecotoxicology – Interactions Among Contaminants and Other Stressors** 414, 415, 416, 417, 418, 419, 420, 421, WP047, WP048, WP049, WP050, WP051, WP052, WP053, WP054, WP055, WP056, WP057
- Aniston PCB Site: Ecological and Human Health Risk Assessment Status and Initial Findings** 77, 78, 79, 80, 81, 82, 83, 84, MP237, MP238, MP239, MP240, MP241
- AQUAFATE – The Fate of Organic Pollutants in Aquatic Environments** 653, 654, 655, 656, 657, 658, 659, 660, RP040, RP041, RP042, RP043, RP044, RP045, RP046, RP047, RP048, RP049
- Aquatic Macrophyte Ecotoxicology (AMEG)** RP132, RP133, RP134, RP135, RP136
- Aquatic Toxicology and Ecology General** RP137, RP138, RP139, RP140, RP141, RP142, RP143, RP144, RP145, RP146, RP147, RP148, RP149, RP150, RP151, RP152, RP153, RP154, RP155, RP156, RP157, RP158, RP159, RP160, RP161, RP162, RP163, RP164, RP165, RP166, RP167, RP168, RP169, RP170, RP171, RP172, RP173, RP174, RP175, RP176, RP177, RP178, RP179, RP180, RP181, RP182, RP183, RP184, RP185, RP186, RP187, RP188, RP189, RP190, RP191, RP192, RP193, RP194, RP195, RP196, RP197, RP198, RP199, RP200, RP201, RP202, RP203, RP204, RP205, RP206, RP207, RP208, RP209, RP210, RP211, RP212, RP213, RP214, RP215, RP216, RP217, RP218, RP219, RP220, RP221, RP222, RP223, RP224, RP225, RP226, RP227, RP228, RP229, RP230, RP232, RP233, RP234, RP235, RP236, RP237, RP238, RP239, RP240, RP241, RP242, RP243, RP244, RP245, RP246, RP247, RP248, RP249, RP250, RP251, RP252, RP253, RP254, RP255, RP256, RP257, RP258, RP259, RP260, RP261, RP262, RP263, RP264, RP265, RP266, RP267, RP268, RP269, RP270, RP271, RP272, RP273, RP274, RP275, RP276, RP277, RP278, RP279, RP280, RP281
- Assessing the Effects and Risks of Biorefinery Products for the Environment** 709, 710, 711, 712, 713, 714
- Assessing the Effects of Major Ions on Aquatic Life and the Role of Water Quality in Altering Major Ion Toxicity** MP037, MP038, MP039, MP040, MP041
- Assessing the Risks of Plant Protection Products to Honey Bees and Other Pollinators** 9, 10, 11, 12, 13, 14, 15, 16 MP062, MP063, MP064, MP065, MP066, MP067, MP068
- Assessment of Human Health Risks Arising from Pharmaceuticals in the Environment** 124, 125, 126, 127, 128, 129, 130, 131, WP155, WP156, WP157
- Atmospheric Chemistry: Transport and Fate of Organic Contaminants** TP144, TP145, TP146, TP147, TP148, TP149
- Atmospheric Chemistry: Transport and Fate of Organic Contaminants – Part 1** 438, 439, 440, 441, 442, 443, 444, 445
- Atmospheric Chemistry: Transport and Fate of Organic Contaminants – Part 2** 534, 535, 536, 537, 538, 539, 540, 541
- Beyond Estrogens and Estrogenicity: Pairing Chemistry and Mode of Action Screening** 17, 18, 19, 20, 21, 22, 23, 24
- Beyond Estrogens and Estrogenicity: Pairing Chemistry and Mode of Action Screening** MP147, MP148, MP149, MP150, MP151, MP152
- Bioaccumulation – Lessons Learned and Challenges Ahead** 203, 204, 205, 206, 207, 208, 209 TP080, TP081, TP082, TP083, TP084, TP085, TP086, TP087, TP088, TP089, TP090, TP091
- Bioaccumulation Science** TP205, TP206, TP207, TP208, TP209, TP210, TP211, TP212, TP213, TP214, TP215, TP216, TP217, TP218
- Bioaccumulative Aquatic Contaminants: Translating Tissue Concentrations in Support of Regulation and Remediation** 669, 670, 671, 672, 673, 674, 676 RP069, RP070, RP071, RP072, RP073, RP074, RP075, RP076, RP077
- Biomonitoring Toxic Chemicals in the Human Body** 367, 368, 369, 370, 371, 372, 373, 374 TP136, TP137, TP138, TP139, TP140, TP141, TP142, TP143
- Black Carbon and Its Role on the (Bio) availability of Organic Contaminants** 755, 756, 757, 758, 759, 760, 761, 762
- Boston Harbor and MA Bay: Critical Issues, Solutions and Environmental Monitoring – Dedicated to Professor Gordon Wallace** RP050, RP051, RP052
- Boston Harbor and MA Bay: Critical Issues, Solutions and Environmental Monitoring – Dedicated to Professor Gordon Wallace – Part 1** 589, 590, 591, 592, 593, 594, 595, 596
- Boston Harbor and MA Bay: Critical Issues, Solutions and Environmental Monitoring – Dedicated to Professor Gordon Wallace – Part 2** 693, 694, 695, 696, 697, 698, 699, 700
- Chemistry and Toxicology of Urban Stormwater Runoff** 723, 724, 725, 726, 727, 728, 729, 730 RP011, RP012, RP013, RP014, RP015, RP016, RP017, RP018, RP019, RP020, RP021, RP022, RP023, RP024, RP025, RP026, RP027
- Chemists in SETAC** TP219, TP220, TP221
- Climate Change Impacts on Contaminant Behavior in the Polar Regions** 63, 64, 65, 66, 67, 68, 69
- Computational Toxicology for Risk Assessment of Chemicals** 677, 678, 679, 680, 681, 682, 683, 684, RP084
- Contaminants of Emerging Concern in Drinking Water: Mixtures, Meaning and Management** 344, 345, 346, 347, 348, 349, 350, 351, TP001, TP002, TP003, TP004, TP005, TP006, TP007, TP008, TP009, TP010, TP011, TP012
- Creating and Using Exposure-Response Relationships from Field Data** 661, 662, 663, 664, 665, 666, 667, 668, RP095, RP096, RP097, RP098, RP099
- Current and Emerging Issues in Drinking-Water Quality** TP078, TP079
- Data Quality – Quality Assurance and Quality Control Issues and Practice** RP036, RP037, RP038, RP039
- Decision Analysis: From Local Needs to Global Applications** 422, 423, 424, 425, 426, 427, 428, 429, WP094
- Dioxins, Furans and Dioxin-like Compounds: Current Developments in Chemistry, Toxicology and Policy** 715, 716, 717, 718, 719, 720, 721, 722 RP060, RP061, RP062, RP063
- Ecological Risk Assessment General** MP168, MP169, MP170, MP171, MP172, MP173, MP174, MP175, MP176, MP177, MP178, MP179, MP180, MP181, MP182, MP183, MP184, MP185, MP186, MP187, MP188, MP189, MP190, MP191, MP192, MP193, MP194, MP195, MP196, MP197, MP198, MP199, MP200, MP201, MP202, MP203, MP204, MP205, MP206, MP207, MP208, MP209, MP210, MP211, MP212, MP213, MP214,
- Ecological Risk Assessment at the Hanford Nuclear Site** 290, 291, 292, 293, 294, 295, 296, 297, TP173, TP174
- Ecosystem Services: From Concept to Application** 739, 740, 741, 742, 743, 744, 745, 746, RP085, RP086, RP087, RP088, RP089
- Ecotoxicity, Fate and Risk Assessment of Materials of Importance to the Military** 383, 384, 385, 386, 387, 388, 389, 390, TP097, TP098, TP099, TP100, TP101, TP102, TP103, TP104
- Ecotoxicology of Nanomaterials** WP158, WP159, WP160, WP161, WP162, WP163, WP164, WP165, WP166, WP167, WP168, WP169, WP170, WP171, WP172, WP173, WP174, WP175, WP176
- Ecotoxicology of Nanomaterials – Part 1** 39, 40, 41, 42, 43, 44, 45, 46
- Ecotoxicology of Nanomaterials – Part 2** 140, 141, 142, 143, 144, 145, 146, 147
- Ecotoxicology of Nanomaterials – Part 3** 621, 623, 624, 625, 626, 627, 628
- Ecotoxicity of Dyes and Their Transformation Products** 148, 149, 150, 151, 152, 153, 154, 155
- EDCs and Pharmaceuticals in the Environment** 298, 299, 300, 301, 302, 303, 304, 305 MP137, MP138, MP139, MP140, MP141, MP142, MP143, MP144, MP145, MP146
- Effects of Global Climate Change on the Foundations and Applications of Environmental Toxicology and Chemistry** 398, 399, 400, 401, 402, 403, 404, 405, WP045, WP046
- Elucidating Sources of Perfluorinated Compounds in the Global Environment** 250, 251, 252, 253, 254, 255, 256, 257 TP052, TP053, TP054, TP055, TP056, TP057, TP058
- Emerging Halogenated Phenolic Chemicals in Biotic and Abiotic Environments** 454, 455, 456, 457, 458, 459, 460, 461, WP025, WP026, WP027
- Environmental Analytical Chemistry, QSPR and QSAR Models** MP001, MP002, MP003, MP004, MP005
- Environmental Chemistry** 542, 543, 544, 545, 546, 547, 548, 549, WP001, WP002, WP003, WP004, WP005, WP006, WP007, WP008, WP009, WP010, WP011, WP012
- Environmental Education** WP103, WP104, WP105, WP106
- Environmental Exposures and Public Health Risks** 180, 181, 182, 183, 184, 185, 186, 187 MP086, MP087, MP088, MP089, MP090
- Environmental Fate and Bioaccumulation of Organic Pollutants in the Marine Environment** 258, 259, 260, 261, 262, 263, 264, 265, TP039, TP040, TP041, TP042, TP043, TP044, TP045, TP046, TP047, TP048, TP049, TP050, TP051
- Environmental Fate and Secondary Pollutants: Can We Provide Proper Scientific Information to Policy Makers?** WP189, WP190, WP191
- Environmental Forensics** 645, 646, 647, 648, 649, 650, 651, 652, RP001, RP002, RP003, RP004
- Environmental Forensics – Historical Reconstruction, Background and Apportionment of Releases** RP034, RP035
- Environmental Metabolomics** 763, 764, 765, 766, 767, 768, 769, 770
- Environmental Monitoring and Impacts of Engineered Nanoparticles** 731, 732, 733, 734, 735, 736, 737, 738, RP078, RP079, RP080, RP081, RP082, RP083
- Environmental or Analytical Chemistry General** TP222, TP223, TP224, TP225, TP226, TP227, TP228, TP229, TP230, TP231, TP232, TP233, TP234, TP235, TP236, TP237, TP238, TP239, TP240, TP241, TP242, TP243, TP244, TP245, TP246, TP247, TP248, TP249, TP250, TP251, TP252, TP253, TP254, TP255, TP256, TP257, TP258, TP259, TP260, TP261, TP262, TP263, TP264, TP265, TP266, TP267, TP268, TP269
- Environmental Risks of Energy Generation** 1, 2, 3, 4, 5, 6, 7, 8, MP242
- Estuarine and Marine Fish Ecotoxicology: Understanding and Linking Impacts at Different Levels of Biological Organization** 47, 48, 49, 50, 51, 52, 53, 54, MP042, MP043, MP044, MP045, MP046, MP047, MP048, MP049, MP050, MP051, MP052, MP053
- Evaluating Human Health and Ecological Risk Assessments and Remediation Decisions: Is the Cure Worse than the Disease?** 478, 479, 480, 481, 483, 484, 485, WP126, WP127, WP128
- Evolution of the Stress Response** 132, 133, 134, 135, 136, 137, 138, 139, MP153
- Fate and Bioaccumulation of Emerging Contaminants in Aquatic and Terrestrial Organisms** 352, 353, 354, 355, 356, 357, 358 TP061, TP062, TP063, TP064, TP065, TP066, TP067, TP068, TP069, TP070, TP071, TP072, TP073, TP074, TP075
- Fate and Effects of Manufactured Nanomaterials in Soils and Sediments** 320, 321, 322, 323, 324, 325, 326, 327 TP182, TP183, TP184, TP185, TP186, TP187, TP188, TP189, TP190
- Fate and Effects of Metals: Aquatic Biological Perspective** WP192, WP193, WP194, WP195, WP196, WP197, WP198, WP199, WP200, WP201, WP202, WP203, WP204, WP205, WP206, WP207, WP208, WP209, WP210, WP211, WP213, WP214, WP215, WP216, WP217, WP218, WP219, WP220, WP221, WP222, WP223, WP224, WP225, WP226, WP227, WP228, WP229, WP230, WP231
- Fate and Effects of Metals: Aquatic Biological Perspective – Part 1** 406, 407, 408, 409, 410, 411, 412, 413
- Fate and Effects of Metals: Aquatic Biological Perspective – Part 2** 502, 503, 504, 505, 506, 507, 508, 509

- Fate and Effects of Metals: Geochemical Perspective** 597, 598, 599, 600, 601, 602, 603, 604, RP121, RP122, RP123, RP124, RP125, RP126
- Fate and Effects of Metals: Regulatory and Risk Assessment Perspective** 226, 227, 228, 229, 230, 231, 232, 233, WP232, WP233, WP234, WP235
- Fate and Effects of Metals: Terrestrial/Wetland Perspective** 701, 702, 703, 704, 705, 706, 707, 708, RP127, RP128, RP129, RP130, RP131
- Fish and Wildlife Investigations** 510, 511, 512, 513, 514, 515, 516, 517, WP107, WP108, WP109, WP110, WP111, WP112, WP113, WP114, WP115, WP116, WP117, WP118, WP119, WP120
- Flame Retardants and Perfluorochemicals in Indoor Environments** 172, 173, 174, 175, 176, 177, 178, 179, MP124, MP125, MP126, MP127
- Fukushima Environmental Crisis** 494, 495, 497, 498, 499, 500, 501
- Fungicides: Occurrence, Environmental Fate and Toxicity** 242, 243, 244, 245, 246, 247, 248, 249, TP175, TP176, TP177, TP178
- Great Lakes Binational Monitoring and Surveillance of Critical Substances and Priority Toxics: Chemicals of Emerging Concern** 164, 165, 166, 167, 168, 169, 170, 171, MP054, MP055, MP056, MP057, MP058
- Green and Sustainable Chemistry: Designing Environmentally Safer Chemicals** 581, 582, 583, 584, 585, 586, 587, 588 RP053, RP054, RP055, RP056, RP057, RP058
- Green Chemistry Strategies: Product Scoring and Evaluation Frameworks – Applications and Possible Enhancements** 685, 686, 687, 688, 689, 690, 691, 692
- Gulf of Mexico Deepwater Horizon/ Macondo 252 Well Incident Special Symposium** TP015, TP016, TP017, TP018, TP019, TP020, TP021, TP022, TP023, TP025, TP026, TP027, TP028, TP029, TP030, TP031, TP032, TP033, TP034, TP035, TP036, TP037, TP038
- Gulf of Mexico Deepwater Horizon/ Macondo 252 Well Incident Special Symposium – Moderated Panel** 109, 110, 111, 112, 113, 114, 115
- Gulf of Mexico Deepwater Horizon/ Macondo 252 Well Incident Special Symposium – Part 1** 210, 211, 212, 213, 214, 215, 216, 217
- Gulf of Mexico Deepwater Horizon/ Macondo 252 Well Incident Special Symposium – Part 2** 391, 392, 393, 394, 395, 396, 397
- History of Environmental Chemistry** 306, 307, 308, 309, 310, 311, 312
- Immunotoxicology** 234, 235, 236, 237, 238, 239, 240, 241 TP114, TP115, TP116, TP117
- Indoor Environments – The Next Frontier** 70, 71, 72, 73, 74, 75, 76, MP154, MP155, MP156
- Integrating Chemical and Biological Approaches to Understand Bioavailability and Enhance Toxicant Identification** 446, 447, 448, 449, 450, 451, 452, 453, WP065, WP066, WP067, WP068, WP069, WP070, WP071, WP072, WP073, WP074, WP075, WP076, WP077
- Invasive Species Risk Assessment** WP058, WP059, WP060, WP061, WP062, WP063, WP064
- Ionic Strength: Impacts on Freshwater and Saltwater Receiving Systems** 274, 275, 276, 277, 278, 279, 280, 281, TP059, TP060
- Killifish (*Fundulus heteroclitus*) and Other Wild Fish Models in Environmental Toxicology: Linking Molecular Mechanism to Higher Levels of Biological Organization** 462, 463, 464, 465, 466, 467, 468, 469, MP071, MP072, MP073, MP074, MP075, MP076, MP077, MP078, MP079, MP080, MP081, MP082
- Lessons Learned and Future Directions of the Ecotoxicity Assays in the EDSP Tier 1 Screening Battery** 116, 117, 118, 119, 120, 121, 122, 123, MP161, MP162, MP163, MP164, MP165, MP166
- Life-Cycle Assessment (LCA)** RP282, RP283, RP284, RP285
- Mercury Cycling and Bioaccumulation in Rivers – Status, Trends and Dynamics** TP093, TP094, TP095, TP096
- Mercury Fate and Biogeochemistry** 31, 32, 33, 34, 35, 36, 37, 38 MP025, MP026, MP027, MP028, MP029, MP030, MP031, MP032, MP033, MP034
- Mercury in the Environment** TP191, TP192, TP193, TP194, TP195, TP196, TP197, TP198, TP199, TP200
- Mercury Toxicity: The Influence of Other Dietary Factors** TP170, TP171, TP172
- Metal Soil Concentration: What Do the Numbers Really Mean** WP021, WP022, WP023, WP024
- Metals** WP236, WP237, WP238, WP239, WP240
- Methods and Models for Ecotoxicological Genomics** 629, 630, 631, 632, 633, 634, 635, 636, RP090, RP091, RP092, RP093, RP094
- Metrology Tools for Studying the Behavior and Effects of Nanomaterials in the Environment and Biological Systems** 266, 267, 268, 269, 270, 271, 272, 273, TP201, TP202, TP203, TP204
- Molecular Indicators: Discovery, Validation and Applications in Aquatic Toxicology/ Ecology** 558, 559, 560, 561, 562, 563, 564, 565, WP135, WP136, WP137, WP138, WP139, WP140, WP141, WP142, WP143, WP144, WP145
- Molecular Toxicology, Toxicogenomics and Biomarkers** 518, 519, 520, 521, 522, 523, 524, 525, WP101, WP102
- Multi-disciplinary Approaches and Case Studies for Characterizing and Monitoring Contaminated Sediment Remediation** 188, 189, 190, 191, 192, 193, 194 MP128, MP129, MP130, MP131, MP132, MP133, MP134, MP135, MP136
- Nanotechnology** RP286, RP287, RP288, RP289, RP290, RP291, RP292, RP293, RP294, RP295
- Neurotoxic Effects in Fish and Wildlife** 336, 337, 338, 339, 340, 341, 342, 343, TP105, TP106, TP107, TP108, TP109, TP110, TP111, TP112, TP113
- New and Alternate (Non) Halogenated Flame Retardants in the Environment** MP006, MP007, MP008, MP009, MP010, MP011, MP012, MP013, MP014, MP015, MP016, MP017, MP018
- New Developments in Environmental Mass Spectrometry and Analytical Chemistry** MP019, MP020, MP021, MP022, MP023, MP024
- New Developments in LCA Impact Assessment** 771, 772, 773, 774, 775, 776, 777, 778, RP112, RP113, RP114, RP115, RP116, RP117, RP118, RP119, RP120
- PAH Exposure and Implications Regarding Human and Ecological Risks** 359, 360, 361, 362, 363, 364, 365, 366, TP150, TP151, TP152, TP153, TP154, TP155, TP156, TP157, TP158, TP159, TP160, TP161, TP162, TP163, TP164
- Pharmaceuticals** TP270, TP271
- Prioritization of Chemicals of Emerging Concern for Environmental Monitoring and Assessment** 637, 638, 639, 640, 641, 642, 643, 644, RP028, RP029, RP030, RP031, RP032
- Recent Developments in Polyfluorinated Compound Research** TP118, TP119, TP120, TP121, TP122
- Regional and Watershed Scale Integrated Risk Assessment** 101, 102, 103, 104, 105, 106, 107, 108, MP167
- Risk Communication: Lessons Learned** 779, 780, 781, 782, 783, 784, 785, 786, RP111
- Risk Management, Remediation or Science Policy General** MP215, MP216, MP217, MP218, MP219, MP220, MP221, MP222, MP223, MP224, MP225, MP226, MP227, MP228, MP229, MP230, MP231, MP232, MP233, MP234, MP235, MP236
- Screening Techniques in Environmental Mass Spectrometry** RP064, RP065, RP066, RP067, RP068
- Sediment** WP241, WP242, WP243, WP244, WP245, WP246, WP247, WP248, WP249, WP250
- SETAC–United Nations Environmental Programme Partnership: Implications for Mercury Pollution, Global Change and the 2013 World Treatise** 282, 283, 284, 285, 286, 287, 288, 289, TP076, TP077
- Soil Ecotoxicology and Risk Assessment** 574, 575, 576, 577, 578, 579, 580 WP121, WP122, WP123, WP124, WP125
- Terrestrial or Wildlife Toxicology and Ecology General** WP251, WP252, WP253, WP254, WP255, WP256, WP257, WP258, WP259, WP260, WP261, WP262, WP263, WP264, WP265, WP266, WP267, WP268, WP269, WP270, WP271, WP272, WP273, WP274, WP275, WP276, WP277, WP278, WP279, WP280, WP281, WP282, WP283, WP284, WP285, WP286
- The Canadian Oil Sands, Part I: Assessing Impacts on the Environment and the Advancement of Oil Sands Reclamation Strategies** 470, 471, 472, 473, 474, 475, 476, 477, WP034, WP035, WP036, WP037, WP038, WP039, WP040, WP041, WP042, WP043, WP044
- The Canadian Oil Sands, Part II: Advancements in the Understanding of the Ecotoxicology of Oil Sands Waste Materials and Analytical Detection Methods** 566, 567, 568, 569, 570, 571, 572, 573 WP028, WP029, WP030, WP031, WP032, WP033
- The Role of Chemical Contamination in the Decline of American Eel** 195, 196, 197, 198, 199, 200, 201, 202, MP069, MP070
- The Role of Ecology in Developing Ecological Production Functions for Ecosystem Services Assessment** MP083, MP084, MP085
- The Use of Adverse Outcome Pathways in Ecotoxicological Hazard of Chemicals in Risk Assessment** 25, 26, 27, 28, 29, 30
- Toxicity and Biodegradation of Petroleum in Arctic Marine Environments** 605, 606, 607, 608, 609, 610, 611, 612, RP033
- Toxicological and Ecotoxicological Impacts of Ocean Acidification** WP129, WP130, WP131, WP132, WP133, WP134
- Trophic Transfer of Bioaccumulative Contaminants** 93, 94, 95, 96, 97, 98, 99, 100, MP035, MP036
- Tropical Ecotoxicology: How Can We Ensure that “Lessons Learned” Are Applied to Emerging Problems in Developing Countries?** MP091, MP092, MP093
- Understanding and Management of Harmful Algal Blooms** WP095, WP096, WP097, WP098, WP099, WP100
- What Do We Know About the Safety of Ingredients in Personal Care Products?** 526, 527, 528, 529, 530, 531, 532, 533 WP013, WP014, WP015, WP016, WP017, WP018, WP019, WP020
- What Is the Current State of the Science on Fate/Exposure/Effects of Pharmaceuticals to Ecological Receptors?** 430, 431, 432, 433, 434, 435, 436, 437, WP146, WP147, WP148, WP149, WP150, WP151, WP152, WP153, WP154
- What’s in My Backyard and How Did It Get There?** 550, 551, 552, 553, 554, 555, 556, 557, WP177, WP178, WP179, WP180
- Wildlife Ecotoxicology: Forensic Approaches** 747, 748, 749, 750, 751, 752, 753, 754, RP059
- You Can Teach an Old Dog New Tricks: Research Applications of Standard Toxicity Tests** 613, 614, 615, 616, 617, 618, 619, 620 RP100, RP101, RP102, RP103, RP104, RP105, RP106, RP107, RP108, RP109, RP110



- Accumulation** 7, 69, 93, 94, 204, 259, 264, 321, 357, 476, 502, 507, 532, 627, 660, 663, 707, MP007, MP023, MP030, MP036, MP116, MP247, TP011, TP067, TP069, TP089, TP102, TP217, TP218, TP225, TP226, TP248, TP252, WP026, WP048, WP054, WP197, WP215, WP216, WP231, WP237, WP262, WP277, RP077, RP129, RP170, RP183, RP229, RP244, RP268, RP295
- Acute toxicity** 11, 41, 136, 139, 152, 154, 278, 314, 315, 329, 330, 333, 361, 394, 395, 406, 410, 413, 469, 472, 477, 506, 520, 522, 564, 575, 578, 584, 609, 692, 701, 713, 729, 764, MP038, MP039, MP041, MP049, MP052, MP079, MP171, MP181, MP212, MP225, TP030, TP232, TP124, TP128, TP161, TP176, TP178, WP043, WP049, WP060, WP065, WP066, WP112, WP145, WP159, WP160, WP165, WP173, WP175, WP200, WP214, WP223, WP225, WP228, WP229, WP257, WP275, RP053, RP102, RP108, RP160, RP172, RP173, RP174, RP195, RP201, RP206, RP215, RP219, RP220, RP246, RP255, RP270, RP271, RP272
- Adsorption** 42, 85, 92, 351, 387, 388, 655, MP026, MP097, MP116, TP010, TP017, TP241, TP263, RP125, RP233
- Ammonia** 162, 421, MP140, WP195, RP147, RP209
- Aquatic toxicity** 18, 21, 29, 39, 44, 45, 47, 51, 53, 54, 78, 104, 116, 121, 125, 134, 138, 142, 143, 147, 148, 151, 154, 201, 210, 218, 223, 224, 227, 238, 241, 246, 247, 258, 275, 276, 278, 279, 280, 295, 312, 314, 316, 317, 318, 319, 334, 340, 341, 342, 355, 361, 375, 376, 381, 383, 391, 392, 393, 408, 410, 411, 413, 419, 434, 448, 453, 464, 467, 468, 469, 484, 502, 504, 505, 507, 509, 561, 563, 564, 565, 566, 567, 568, 570, 581, 606, 607, 608, 611, 613, 614, 615, 618, 619, 624, 625, 627, 632, 634, 662, 678, 683, 686, 692, 737, 768, 769, 780, MP005, MP009, MP031, MP037, MP039, MP040, MP041, MP043, MP046, MP047, MP049, MP050, MP051, MP054, MP059, MP061, MP069, MP071, MP073, MP075, MP077, MP078, MP079, MP080, MP081, MP082, MP092, MP093, MP138, MP140, MP148, MP149, MP151, MP152, MP157, MP160, MP161, MP166, MP174, MP181, MP198, TP008, TP022, TP027, TP030, TP032, TP059, TP060, TP106, TP110, TP112, TP114, TP123, TP125, TP126, TP127, TP128, TP129, TP131, TP133, TP134, TP179, WP017, WP025, WP028, WP032, WP034, WP035, WP036, WP040, WP041, WP042, WP044, WP045, WP055, WP057, WP059, WP064, WP071, WP072, WP077, WP083, WP086, WP087, WP088, WP096, WP097, WP103, WP108, WP119, WP120, WP133, WP135, WP138, WP140, WP141, WP142, WP144, WP146, WP150, WP153, WP162, WP163, WP166, WP168, TP168, WP170, WP171, WP173, WP192, WP193, WP195, WP200, WP201, WP202, WP203, WP204, RP204, WP205, WP206, WP207, WP208, WP209, WP210, WP211, WP216, WP219, WP221, WP225, WP227, WP231, WP234, RP025, RP033, RP037, RP049, RP070, RP071, RP080, RP081, RP084, RP090, RP091, RP094, RP097, RP100, RP101, RP102, RP103, RP106, RP108, RP109, RP132, RP133, RP137, RP139, RP140, RP141, RP143, RP146, RP149, RP153, RP155, RP157, RP165, RP167, RP168, RP169, RP171, RP172, RP173, RP174, RP175, RP176, RP177, RP178, RP181, RP186, RP187, RP189, RP190, RP191, RP195, RP199, RP201, RP203, RP206, RP207, RP211, RP212, RP216, RP217, RP218, RP219, RP225, RP228, RP230, RP232, RP234, RP236, RP237, RP238, RP239, RP240, RP243, RP245, RP250, RP252, RP258, RP260, RP261, RP262, RP267, RP269, RP271, RP276, RP278, RP280, RP281, RP292, RP294, RP295
- Atrazine** 240, 419, 444, 524, 635, 754, MP232, RP051, RP095, RP097, RP099, RP252, RP276
- Behavior** 68, 105, 175, 222, 256, 300, 310, 318, 337, 340, 343, 356, 493, 684, 747, MP016, MP052, MP124, MP137, MP145, TP016, TP038, TP107, TP109, TP110, TP111, TP113, TP148, TP162, TP249, TP263, TP271, WP011, WP046, WP047, WP063, WP070, WP085, WP103, WP105, WP119, WP123, WP149, WP153, WP164, WP169, WP190, WP216, WP222, WP226, RP004, RP009, RP034, RP072, RP073, RP076, RP173, RP270
- Bioaccumulation** 31, 33, 50, 67, 70, 76, 77, 90, 93, 95, 96, 97, 98, 99, 100, 143, 144, 145, 146, 150, 156, 163, 165, 167, 168, 169, 171, 173, 175, 189, 193, 195, 197, 202, 203, 204, 207, 208, 209, 251, 252, 257, 262, 263, 265, 283, 284, 292, 297, 309, 328, 336, 352, 353, 356, 360, 362, 371, 384, 396, 450, 460, 474, 497, 498, 503, 504, 506, 508, 509, 511, 546, 550, 559, 587, 594, 596, 605, 623, 641, 669, 670, 671, 672, 673, 674, 676, 703, 704, 721, 759, 764, MP007, MP012, MP015, MP016, MP031, MP032, MP033, MP034, MP035, MP054, MP056, MP058, MP092, MP109, MP122, MP132, MP157, MP160, MP174, MP192, MP193, MP201, MP230, MP239, TP039, TP043, TP045, TP048, TP049, TP051, TP052, TP061, TP063, TP064, TP065, TP066, TP068, TP071, TP072, TP074, TP075, TP080, TP081, TP082, TP083, TP084, TP085, TP086, TP087, TP089, TP090, TP091, TP093, TP096, TP136, TP141, TP170, TP171, TP172, TP181, TP190, TP195, TP196, TP205, TP206, TP207, TP208, TP209, TP210, TP211, TP212, TP213, TP214, TP215, TP216, TP217, TP223, TP235, TP240, TP247, TP253, TP268, WP010, WP111, WP121, WP154, WP166, WP177, WP193, WP217, WP218, WP222, WP228, WP234, WP255, WP256, WP260, WP263, WP271, WP276, WP280, RP050, RP065, RP067, RP068, RP072, RP073, RP074, RP075, RP076, RP077, RP123, RP130, RP131, RP142, RP150, RP162, RP179, RP182, RP188, RP194, RP205, RP226, RP245, RP248, RP291
- Bioavailability** 24, 44, 45, 59, 85, 87, 88, 89, 92, 94, 145, 150, 190, 192, 210, 213, 226, 228, 229, 231, 266, 279, 322, 323, 337, 354, 355, 361, 389, 399, 406, 409, 410, 411, 446, 447, 548, 576, 597, 600, 603, 604, 607, 609, 660, 667, 700, 703, 755, 757, 758, 759, 761, 766, MP021, MP094, MP095, MP096, MP097, MP098, MP099, MP100, MP101, MP103, MP107, MP118, MP131, MP159, MP179, MP189, MP212, TP021, TP031, TP065, TP070, TP076, TP077, TP088, TP094, TP098, TP099, TP150, TP152, TP155, TP186, TP187, TP190, TP218, TP227, TP238, TP246, TP263, WP002, WP003, WP021, WP065, WP066, WP067, WP068, WP069, WP148, WP162, WP169, WP178, WP204, WP205, WP219, WP227, WP235, WP236, WP238, WP241, WP255, WP256, RP006, RP009, RP036, RP072, RP073, RP121, RP122, RP123, RP128, RP129, RP155, RP186, RP200, RP235, RP273, RP275
- Bioconcentration** 42, 93, 124, 204, 205, 206, 208, 265, 311, 433, 531, 583, 654, 716, TP062, TP066, TP075, TP084, TP088, TP091, TP124, TP195, TP205, TP211, TP212, TP240, WP092, WP110, WP129, RP188, RP195
- Biodegradation** 154, 211, 214, 216, 250, 265, 303, 543, 586, 610, 611, 642, 688, 689, 691, 692, 709, 718, 720, MP189, MP231, TP015, TP049, TP055, TP219, WP028, WP155, RP005, RP057, RP058
- Biomonitoring** 13, 51, 53, 76, 102, 106, 114, 134, 169, 172, 173, 177, 178, 179, 198, 257, 263, 296, 297, 335, 360, 367, 369, 370, 372, 373, 383, 455, 456, 474, 515, 516, 549, 561, 593, 594, 644, 665, 666, 667, 695, 712, 769, MP007, MP043, MP050, MP053, MP054, MP076, MP077, MP113, MP119, MP122, MP127, MP185, MP187, MP209, MP213, TP025, TP051, TP066, TP073, TP074, TP075, TP129, TP136, TP137, TP138, TP139, TP142, TP143, TP164, TP209, TP214, TP216, TP217, TP229, TP259, WP008, WP025, WP050, WP051, WP071, WP108, WP137, WP141, WP192, WP266, RP010, RP014, RP034, RP067, RP073, RP075, RP093, RP095, RP099, RP154, RP175, RP182, RP194, RP196, RP257, RP258, RP259, RP266, RP278, RP279
- Bioremediation** 76, 215, 286, 289, 354, 392.5, 471, 610, MP021, MP097, MP129, MP132, MP136, MP218, MP231, TP163, WP044, WP276
- Biotransformation** 37, 93, 100, 132, 133, 203, 206, 208, 250, 253, 300, 354, 358, 456, 464, 562, 606, MP010, MP014, MP026, MP028, MP102, MP137, MP158, MP232, TP043, TP044, TP049, TP055, TP064, TP069, TP080, TP084, TP089, TP095, TP124, TP202, TP257, WP196, RP047, RP127, RP187, RP244
- Case study** 8, 27, 35, 57, 174, 180, 199, 210, 228, 237, 262, 306, 308, 310, 312, 365, 380, 498, 516, 548, 549, 551, 552, 556, 589, 592, 612, 645, 648, 649, 664, 695, 720, 722, 739, 740, 747, 749, 751, 752, 778, 779, 780, 781, 782, 784, 786, MP014, MP084, MP089, MP090, MP130, MP132, MP133, MP143, MP155, MP157, MP162, MP204, MP222, MP225, MP226, MP228, MP233, MP235, TP002, TP019, TP200, TP022, TP028, TP038, TP253, WP050, WP059, WP093, WP100, WP106, WP108, WP109, WP128, WP252, RP014, RP033, RP041, RP043, RP060, RP072, RP105, RP117, RP193, RP283
- Chemical signalling** 26, 222, 363, 477, 565, TP014, TP015, TP103, WP149
- Chronic toxicity** 11, 144, 195, 236, 278, 335, 375, 435, 448, 464, 471, 477, 506, 570, 614, 619, 625, 704, 712, MP039, MP041, MP048, MP091, MP148, MP181, MP199, MP237, MP245, TP027, TP097, TP139, TP140, TP154, TP179, TP193, WP055, WP077, WP130, WP146, WP167, WP176, WP194, WP215, WP217, WP218, WP224, WP229, RP070, RP071, RP100, RP104, RP109, RP110, RP140, RP156, RP160, RP161, RP172, RP206, RP215, RP219, RP227, RP236, RP260, RP264, RP276
- Climate** 63, 64, 65, 66, 187, 262, 283, 398, 399, 400, 402, 403, 405, 427, 539, 697, 711, 768, 782, MP033, MP167, TP060, WP045, WP046, WP058, WP095, WP105, WP130, WP131, WP132, RP114, RP210, RP250, RP281
- Cytotoxicity** 286, 416, 621, 623, 680, 711, 713, MP200, TP114, TP117, WP102
- Decision analysis** 3, 5, 6, 102, 209, 422, 424, 425, 481, 733, 744, 745, 747, 786, MP168, MP214, TP018, TP158, WP094, WP100, RP089
- Degradation** 37, 92, 151, 171, 212, 347, 532, 540, 545, 582, 641, 645, 646, 653, 684, 689, 710, MP145, MP232, TP017, TP023, TP070, TP118, TP124, TP167, TP228, TP265, WP004, WP017, WP175, WP189, WP191, RP004, RP027, RP043, RP046, RP047, RP233
- Depuration** 62, 143, 252, MP112, TP091, TP207, RP006
- Desorption** 387, 388, 552, 660, 756, MP017, RP126, RP223
- Development** 15, 18, 36, 40, 84, 157, 163, 234, 266, 331, 334, 338, 363, 381, 420, 474, 521, 564, 568, 572, 629, 630, 678, 717, 763, MP003, MP022, MP073, MP075, MP108, MP118, MP149, MP236, MP245, TP033, TP034, TP053, TP058, TP107, TP108, TP116, TP117, TP125, TP127, TP131, TP246, TP256, WP030, WP057, WP104, WP137, WP186, WP188, WP269, WP270, WP282, RP025, RP084, RP091, RP103, RP113, RP116, RP152, RP161, RP177, RP199, RP214, RP247, RP282
- Dioxins** 27, 86, 94, 182, 196, 199, 200, 462, 466, 513, 553, 630, 715, 717, 718, 719, 720, 721, 722, 755, 758, MP070, MP075, MP114, MP154, MP192, MP201, WP248, WP253, WP266, RP060, RP061, RP062, RP063, RP187, RP254
- Ecological risk assessment** 10, 11, 12, 14, 27, 28, 29, 30, 48, 51, 59, 77, 78, 82, 96, 97, 100, 101, 102, 103, 104, 105, 106, 108, 109, 125, 138, 149, 187, 191, 192, 195, 196, 200, 207, 244, 284, 290, 292, 293, 294, 295, 296, 312, 328, 361, 363, 380, 384, 385, 386, 391, 398, 403, 425, 479, 483, 484, 486, 487, 488, 489, 490, 491, 493, 495, 501, 510, 511, 517, 518, 526, 527, 528, 529, 574, 575, 597, 599, 607, 613, 617, 619, 638, 644, 664, 667, 677, 678, 679, 680, 681, 682, 684, 685, 690, 708, 719, 724, 737, 739, 740, 741, 742, 743, 744, 746, 749, 767, 779, 781, 782, 783, 785, MP006, MP020, MP029, MP036, MP042, MP050, MP052, MP060, MP062, MP064, MP065, MP070, MP085, MP093, MP123, MP133, MP134, MP135, MP138, MP139, MP143, MP168, MP169, MP170, MP171, MP172, MP173, MP175, MP176, MP177, MP178, MP179, MP180, MP181, MP182, MP184, MP185, MP186, MP187, MP189, MP190, MP192, MP193, MP194, MP195, MP198, MP199, MP201, MP202, MP204, MP205, MP206, MP207, MP208, MP210, MP211, MP212, MP213, MP216, MP222, MP224, MP228, MP233, WP236, MP238, MP239, TP031, TP081, TP084, TP099, TP150, TP151, TP165, TP166, TP170, TP173, TP174, TP200, TP210, TP252, TP259, TP270, WP017, WP042, WP048, WP052, WP058, WP059, WP061, WP062, WP064, WP074, WP077, WP078, WP079, WP080, WP081, WP082, WP083, WP084, WP085, WP087, WP088, WP090, WP091, WP092, WP093, WP095, WP107, WP110, WP117, WP121, WP122, WP127, WP128, WP130, WP155, WP185, WP189, WP209, WP239, WP242, WP250, WP252, WP257, WP259, WP260, WP268, WP281, RP010, RP059, RP063, RP069, RP084, RP085, RP087, RP093, RP097, RP098, RP121, RP122, RP132, RP135, RP137, RP181, RP239, RP250, RP251, RP258, RP270, RP271, RP283, RP284, RP290
- Ecotoxicology** 12, 14, 15, 16, 26, 27, 28, 29, 32, 33, 39, 41, 43, 44, 45, 46, 48, 49, 50, 52, 109, 117, 126, 132, 134, 138, 141, 142, 152, 156, 200, 202, 218, 219, 221, 222, 223, 246, 247, 248, 267, 275, 276, 281, 295, 312, 313, 319, 320, 323, 324, 325, 330, 335, 336, 339, 360, 379, 385, 386, 391, 392.5, 398, 401, 404, 407, 415, 417, 419, 420, 421, 430, 431, 434, 451, 452, 463, 468, 470, 486, 490, 491, 492, 508, 512, 514, 516, 517, 518, 523, 525, 558, 560, 564, 567, 570, 574, 576, 577, 579, 580, 588, 598, 606, 608, 609, 613, 617, 618, 620, 624, 625, 628, 631, 632, 633, 637, 663, 666, 668, 681, 682, 687, 691, 692, 694, 708, 709, 710, 712, 713, 719, 727, 747, 748, 749, 750, 753, 759, 766, 767, 769, 770, 780, MP001, MP009, MP014, MP015, MP016, MP020, MP033, MP043, MP047, MP048, MP052, MP059, MP060, MP061, MP062, MP063, MP065, MP068, MP070, MP072, MP075, MP077, MP078, MP080, MP081, MP082, MP085, MP091, MP092, MP093, MP098, MP112, MP144, MP147, MP150, MP164, MP165, MP182, MP187, MP191, MP196, MP198, MP203, MP207, MP213, MP247, TP027, TP105, TP109, TP125, TP127, TP129, TP132, TP132, TP133, TP135, TP161, TP167, TP174, TP187, TP193, TP194, TP196, TP213, TP219, WP018, WP020, WP030, WP033, WP035, WP040, WP045, WP048, WP049, WP051, WP052, WP053, WP058, WP064, WP079, WP089, WP090, WP093, WP095, WP097, WP106, WP110, WP111, WP114, WP116, WP118, WP123, WP124, WP133, WP134, WP138, WP139, WP140, WP143, WP144, WP150, WP152, WP158, WP159, WP160, WP161, WP162, MP163, WP165, WP168, WP173, WP182, WP184, WP185, WP186, WP187, WP199, WP209, WP222, WP238,



## KEYWORD INDEX

WP240, WP241, WP248, WP251, WP254, WP255, WP256, WP257, WP258, WP259, WP260, WP261, WP262, WP263, WP267, WP268, WP272, WP274, WP281, WP283, WP284, RP011, RP012, RP015, RP033, RP037, RP053, RP055, RP057, RP059, RP078, RP094, RP095, RP098, RP099, RP101, RP103, RP105, RP131, RP132, RP134, RP138, RP141WP142, RP146, RP149, RP150, RP152, RP159, RP160, RP161, RP169, RP174, RP175, RP176, RP177, RP183, RP189, RP191, RP193, RP200, RP201, RP207, RP209, RP210, RP211, RP212, RP213, RP215, RP220, RP226, RP227, RP228, RP232, RP237, RP241, RP242, RP248, RP252, RP253, RP256, RP258, RP260, RP261, RP263, RP264, RP273, RP275, RP277, RP278, RP288, RP290

**Elimination** 174, 178, 373, TP010, TP012, RP060, RP117, RP129, RP295

**Endocrine disruption** 17, 18, 19, 20, 21, 22, 26, 49, 54, 116, 117, 118, 119, 120, 121, 122, 123, 137, 174, 179, 220, 221, 223, 224, 234, 298, 299, 301, 302, 315, 331, 332, 334, 342, 345, 354, 355, 372, 379, 382, 383, 418, 431, 432, 435, 449, 454, 455, 456, 457, 459, 461, 515, 522, 523, 524, 538, 558, 632, 637, 638, 656, 679, 727, 752, 754, MP015, MP044, MP046, MP047, MP073, MP077, MP078, MP081, MP111, MP119, MP120, MP125, MP127, MP137, MP138, MP139, MP140, MP141, MP142, MP144, MP146, MP147, MP150, MP151, MP152, MP161, MP162, MP163, MP164, MP165, MP166, MP194, MP200, MP209, MP211, TP004, TP006, TP007, TP010, TP011, TP013, TP039, TP043, TP068, TP109, TP115, TP116, TP117, TP121, TP123, TP130, TP136, TP140, TP143, TP229, TP241, TP245, TP251, TP252, TP256, TP257, WP005, WP007, WP051, WP052, WP055, WP075, WP082, WP084, WP098, WP104, WP136, WP137, WP138, WP157, WP188, WP189, WP261, WP269, WP270, WP279, WP282, RP011, RP012, RP014, RP032, RP038, RP066, RP096, RP146, RP148, RP151, RP152, RP165, RP177, RP179, RP204, RP218, RP224, RP228, RP240, RP247, RP253, RP263, RP274

**Genotoxicity** 133, 136, 139, 148, 155, 315, 316, 319, 363, 464, 519, 520, 521, 522, 621, 630, 636, MP079, MP203, TP163, WP011, WP139, WP172, WP176, WP267, RP093, RP279

**Ground water** 127, 183, 304, 350, 543, 612, 645, 648, 732, 777, MP102, MP106, MP115, MP134, MP135, MP136, MP186, TP056, TP102, TP118, TP122, TP261, TP262, RP003, RP016, RP019, RP260, RP080, RP082, RP083

**Growth** 502, 503, 514, 521, 618, 748, 763, MP034, TP097, TP182, WP084, WP144, WP270, RP098, RP104, RP110, RP132, RP133, RP140, RP166, RP247, RP261

**Herbicides** 244, 444, 445, 545, 754, 762, MP019, MP024, MP059, MP111, MP148, MP163, MP178, MP183, MP194, MP245, TP126, TP127, TP149, TP254, WP051, WP091, WP096, WP104, WP144, WP281, RP133, RP135, RP157, RP202, RP252, RP272

**Hormesis** TP097, RP148

**Human health** 2, 7, 64, 70, 71, 72, 80, 109, 127, 130, 149, 155, 172, 173, 175, 178, 181, 184, 187, 288, 304, 344, 349, 364, 365, 366, 368, 369, 370, 371, 373, 374, 383, 389, 390, 396, 402, 454, 455, 456, 457, 459, 479, 480, 494, 495, 543, 585, 598, 629, 669, 686, 687, 705, 722, 776, 778, 781, 782, 783, 784, 785, MP006, MP019, MP086, MP087, MP088, MP124, MP125, MP126, MP155, MP156, MP157, MP174, MP193, MP197, MP199, MP200, MP220, MP230, MP235, MP236, TP004, TP009, TP033, TP074, TP121, TP138, TP139, TP141, TP142, TP154, TP155, TP156, TP160, TP170, TP200, TP225, TP226, TP229, TP234, TP239, TP255, TP257, TP271, WP006, WP007, WP011, WP103, WP126, WP156, WP191, WP239, RP037, RP054, RP056, RP067, RP078, RP229, RP239, RP254

**Immunotoxicity** 234, 235, 236, 237, 238, 239, 240, 241, 417, 418, 472, 513, 566, TP115, TP116, TP117, TP184, TP185, TP188, TP191, WP028, WP113, RP037, RP286

**In situ** 61, 86, 95, 148, 277, 296, 355, 407, 566, 727, 730, 760, 761, MP091, MP105, MP231, MP241, WP071, RP051, RP061, RP236

**Insecticides** 14, 15, 72, 119, 334, 340, 441, 547, 665, 681, 725, 726, 748, MP067, MP118, TP050, TP078, TP113, TP138, TP149, WP086, WP145, WP286, RP087, RP232, RP240

**Landscape** 67, 101, 104, 404, 405, 504, 542, 714, MP009, MP029, MP084, MP229, WP078, RP021, RP115, RP239

**Life-cycle assessment** 3, 429, 471, 523, 598, 685, 706, 736, 771, 772, 773, 774, 775, 776, 777, 778, MP221, MP233, WP152, RP018, RP085, RP113, RP114, RP115, RP116, RP117, RP118, RP120, RP166, RP192, RP282, RP283, RP284, RP285

**Mesocosm** 46, 247, 419, 431, 434, 533, 665, TP202, WP004, RP088, RP097, RP135

**Metabolism** 53, 133, 135, 202, 206, 218, 220, 225, 253, 313, 317, 373, 374, 408, 412, 455, 457, 468, 562, 679, 763, 764, 767, 768, 770, MP010, MP113, MP119, MP246, TP062, TP080, TP089, TP133, TP257, WP026, WP027, WP037, WP133, WP151, WP202, WP203, RP127, RP214, RP244, RP285

**Metalloids** 31, 351, 465, 502, 673, TP171, WP193, WP211, RP071, RP072, RP121, RP124, RP145

**Metals** 32, 33, 37, 38, 53, 55, 58, 94, 95, 96, 134, 156, 161, 186, 226, 227, 228, 229, 230, 231, 232, 233, 282, 283, 285, 288, 289, 314, 320, 336, 350, 378, 381, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 468, 476, 503, 504, 505, 506, 507, 509, 510, 512, 554, 559, 560, 576, 596, 597, 598, 599, 600, 601, 602, 603, 604, 631, 635, 652, 663, 668, 671, 672, 698, 699, 700, 701, 702, 704, 705, 706, 707, 708, 738, 774, 775, 777, MP005, MP023, MP027, MP028, MP030, MP031, MP032, MP036, MP061, MP080, MP092, MP102, MP109, MP110, MP117, MP158, MP160, MP160, MP212, MP241, MP242, TP072, TP076, TP093, TP095, TP096, TP101, TP103, TP108, TP170, TP171, TP172, TP174, TP180, TP191, TP193, TP194, TP196, TP197, TP199, TP200, TP201, TP209, TP213, TP214, TP217, TP230, TP243, TP253, TP264, WP006, WP021, WP023, WP024, WP029, WP038, WP039, WP040, WP041, WP045, WP047, WP053, WP071, WP107, WP112, WP116, WP117, WP129, WP131, WP138, WP163, WP164, WP168, WP177, WP184, WP188, WP192, WP194, WP195, WP196, WP197, WP198, WP199, WP200, WP201, WP202, WP203, WP204, WP205, WP206, WP209, WP210, WP211, WP214, WP215, WP216, WP217, WP218, WP219, WP220, WP221, WP222, WP223, WP224, WP225, WP226, WP227, WP229, WP230, WP231, WP232, WP233, WP234, WP236, WP237, WP238, WP239, WP240, WP262, WP267, WP274, WP276, WP277, WP280, WP283, RP002, RP017, RP031, RP069, RP072, RP074, RP075, RP076, RP102, RP108, RP122, RP123, RP126, RP128, RP130, RP131, RP134, RP142, RP150, RP155, RP164, RP168, RP169, RP172, RP173, RP176, RP181, RP183, RP186, RP207, RP216, RP217, RP225, RP235, RP242, RP243, RP245MP247, RP248, RP250, RP255, RP264, RP268, RP273

**Microcosm** 44, 46, 417, 508, MP223, WP020, RP135

**Mixture toxicity** 145, 195, 199, 200, 223, 312, 331, 365, 394, 395, 413, 437, 452, 519, 527, 569, 570, 607, 635, 643, 665, 667, 678, 680, 696, 752, MP020, MP037, RP108, MP148, MP158, MP225, TP007, TP008, TP022, TP026, TP031, TP032, TP160, TP161, TP168, WP068, WP076, WP119, WP129, WP149, WP157, WP225, RP013, RP018, RP084, RP138, RP141, RP144, RP148, RP153, RP202, RP210, RP211, RP212, RP220, RP221, RP238, RP245, RP265, RP269, RP292

**Monitoring** 48, 55, 57, 61, 99, 105, 107, 114, 138, 158, 164, 166, 167, 175, 184, 211, 243, 244, 255, 256, 277, 356, 366, 371, 396, 445, 458, 473, 515, 536, 537, 538, 541, 543, 559, 569, 571, 589, 590, 591, 592, 601, 605, 611, 637, 644., 676, 693, 695, 724, 725, 736, MP004, MP138, MP021, MP024, MP033, MP051, MP058, MP103, MP104, MP105, MP106, MP111, MP118, MP123, MP128, MP130, MP154, MP155, MP173, TP004, TP005, TP009, TP023, TP028, TP039, TP040, TP042, TP047, TP048, TP052, TP056, TP058, TP075, TP076, TP078, TP081, TP118, TP119, TP146, TP148, TP151, TP175, TP196, TP199, TP200, TP225, TP226, TP233, TP239, TP242, TP248, TP253, TP260, TP262, TP266, TP269, WP006, WP007, WP010, WP023, WP073, WP180, WP190, WP243, RP001, RP004, RP008, RP010, RP028, RP039, RP041, RP061, RP064, RP068, RP074, RP106, RP107, RP184, RP185, RP198, RP230, RP262, RP279, RP293

**Multimedia** 63, 73, 74, 76, 204, 265, 309, 312, 534, 535, 539, 540, 556, 557, 654, 773, 776, MP214, TP049, TP199, TP231, TP236, WP019, WP046, RP043

**Mutagenicity** 133, 136, 139, 148, 151, 180, 331, WP076

**Nanomaterials** 39, 40, 41, 42, 43, 44, 45, 46, 140, 141, 142, 143, 144, 145, 146, 147, 220, 239, 266, 267, 268, 269, 270, 271, 272, 273, 286, 320, 321, 322, 323, 324, 325, 326, 327, 349, 376, 521, 579, 621, 623, 625, 626, 628, 731, 732, 733, 734, 735, 736, 737, 738, MP082, MP116, TP065, TP098, TP183, TP185, TP186, TP187, TP188, TP189, TP190, TP201, TP202, TP203, TP204, TP244, TP249, WP001, WP012, WP158, WP159, WP160, WP162, WP163, WP164, WP165, WP166, WP167, WP168, WP169, WP170, WP171, WP172, WP174, WP175, WP176, WP249, WP265, WP268, RP006, RP007, RP009, RP078, RP080, RP081, RP082, RP083, RP286, RP287, RP289, RP290, RP291, RP292, RP294, RP295

**Natural resource damage** 1, 210, 274, 393, 397, 400, 404, 424, 427, 510, 517, 742, MP185, MP202, TP015, TP020, TP025, TP027, TP035, TP038, TP224, TP252, WP001, WP081, WP096, WP109, WP111, WP116, WP120, WP171, WP250, WP252, RP050, RP136, RP276

**Nutrients** 161, 162, 277, 421, 542, 590, 592, 595, 697, WP099, RP020, RP052, RP088, RP137, RP235, RP244, RP267

**Partitioning** 13, 42, 59, 69, 74, 75, 76, 89, 91, 157, 163, 213, 309, 351, 388, 399, 439, 486, 536, 537, 540, 546, 602, 656, 673, MP002, MP018, MP121, MP193, TP017, TP020, TP031, TP066, TP100, TP102, TP120, TP145, TP212, TP218, TP227, TP238, TP249, TP267, WP009, WP067, WP070, WP148, WP217, WP218, RP223

**Passive sampling** 61, 62, 71, 76, 85, 91, 170, 205, 244, 259, 309, 344, 345, 441, 446, 449, 450, 475, 539, 551, 557, 696, 757, 760, MP055, MP099, MP101, MP104, MP107, MP155, TP147, TP218, TP227, TP233, TP246, TP269, WP002, WP065, WP066, WP067, WP068, WP069, WP179

**Persistent** 62, 63, 64, 65, 66, 67, 76, 100, 107, 163, 167, 170, 171, 172, 174, 176, 198, 203, 207, 212, 235, 236, 254, 257, 259, 303, 312, 356, 370, 374, 399, 438, 442, 443, 457, 458, 460, 476, 534, 536, 537, 539, 541, 551, 552, 556, 581, 583, 587, 641, 655, 656, 657, 756, 770, MP007, MP013, MP018, MP053, MP056, MP057, MP087, MP103, MP104, MP114, MP115, MP122, MP127, MP142, TP005, TP039, TP040, TP043, TP050, TP052, TP057, TP058, TP061, TP063, TP073, TP074, TP079, TP120, TP144, TP146, TP147, TP162, TP223, TP231, TP236, TP258, TP261, TP264, WP005, WP010, WP025, WP032, WP046, WP102, WP115, WP179, WP199, WP244, WP246, WP272, RP035, RP040, RP043, RP046, RP064, RP064, RP105, RP243, RP260

**Personal care products** 83, 99, 125, 158, 165, 261, 303, 318, 346, 347, 358, 430, 432, 433, 435, 440, 528, 529, 530, 531, 532, 533, 540, 541, 582, 587, 643, 657, 690, MP120, MP146, MP162, TP003, TP061, TP063, TP079, TP115, TP141, TP143, TP145, TP231, TP236, TP265, WP009, WP013, WP014, WP016, WP017, WP018, WP019, WP020, WP147, RP028, RP029, RP040, RP047, RP049, RP068, RP139, RP144, RP171, RP182, RP184, RP194, RP206, RP275

**Pesticide** 9, 11, 12, 13, 14, 16, 30, 51, 62, 66, 69, 72, 88, 118, 120, 123, 158, 176, 234, 240, 242, 243, 245, 246, 247, 249, 258, 262, 263, 279, 311, 332, 340, 369, 415, 417, 438, 442, 444, 447, 490, 491, 511, 547, 575, 645, 654, 724, 726, 750, 773, MP003, MP024, MP044, MP059, MP060, MP062, MP063, MP064, MP068, MP080, MP088, MP091, MP095, MP096, MP108, MP122, MP146, MP166, MP169, MP171, MP175, MP176, MP178, MP182, MP187, MP184, MP191, MP195, MP206, MP229, TP040, TP059, TP063, TP106, TP111, TP115, TP124, TP126, TP133, TP138, TP144, TP149, TP177, TP178, TP179, TP223, TP258, TP259, TP264, WP025, WP054, WP065, WP066, WP080, WP086, WP088, WP102, WP104, WP115, WP141, WP259, WP281, WP284, RP013, RP018, RP026, RP027, RP068, RP091, RP133, RP153, RP174, RP175, RP195, RP197, RP200, RP211, RP229, RP232, RP234, RP240, RP270, RP271

**Pharmaceuticals** 124, 125, 126, 127, 130, 131, 159, 264, 298, 300, 304, 305, 341, 346, 347, 357, 430, 432, 433, 434, 436, 437, 461, 531, 538, 582, 586, 635, 637, 638, 639, 658, 753, MP045, MP072, MP103, MP115, MP116, MP120, MP145, MP149, MP168, TP003, TP004, TP008, TP012, TP062, TP069, TP070, TP079, TP109, TP110, TP123, TP166, TP167, TP168, TP169, TP210, TP215, TP250, TP260, TP270, TP271, WP004, WP146, WP147, WP148, WP149, WP150, WP151, WP152, WP153, WP154, WP156, WP157, WP242, RP028, RP030, RP032, RP044, RP045, RP046, RP064, RP144, RP161, RP171, RP188, RP209, RP210, RP222, RP237, RP238

**Policy analysis** 1, 8, 118, 282, 285, 288, 289, 364, 500, 717, 745, 746, 750, 779, MP029, MP089, MP090, MP226, MP228, MP230, TP155, TP159, WP105

**Regulation** 1, 12, 20, 58, 90, 118, 120, 233, 282, 285, 288, 328, 330, 385, 422, 469, 486, 487, 614, 644, 652, 664, 674, 717, 739, 751, 753, MP006, MP062, MP065, MP144, MP166, MP205, MP222, MP230, TP033, TP080, TP082, TP088, TP091, TP096, TP124, TP154, TP165, WP080, WP083, WP145, WP204, WP232, WP243, RP163, RP262

**Remediation** 8, 86, 92, 181, 183, 185, 191, 192, 194, 291, 294, 470, 472, 480, 481, 483, 501, 544, 568, 648, 750, 759, 760, MP096, MP101, MP110, MP131, MP133, MP134, MP215, MP216, MP223, MP224, MP227, MP232, MP234, TP101, TP158, TP241, WP042, WP093, WP126, RP001, RP019, RP075, RP083, RP088, RP089, RP147

**Reproduction** 20, 29, 54, 119, 144, 147, 202, 224, 299, 380, 382, 431, 469, 514, 574, 616, 632, 694, 752, 754, MP045, MP047, MP072, MP123, MP142, MP149, MP150, MP209, MP240, TP038, TP097, TP121, TP123, WP057, WP113, WP115, WP182, WP188, WP221, WP251, WP256, WP261, WP270, WP279, RP070, RP096, RP098, RP104, RP151, RP152, RP166, RP168, RP179, RP212, RP247, RP274

**Risk assessment** 1, 2, 3, 4, 6, 7, 8, 9, 15, 16, 25, 26, 27, 30, 43, 56, 58, 71, 79, 80, 81, 83,

- 104, 107, 121, 126, 129, 130, 150, 180, 181, 184, 185, 187, 189, 191, 215, 217, 230, 231, 233, 266, 283, 284, 297, 302, 330, 364, 365, 371, 372, 377, 378, 389, 390, 400, 402, 403, 408, 412, 426, 427, 428, 429, 435, 436, 437, 478, 479, 480, 485, 489, 494, 498, 499, 503, 512, 563, 577, 585, 609, 620, 629, 638, 639, 642, 662, 670, 676, 677, 685, 689, 712, 719, 722, 733, 735, 745, 748, 774, 779, 780, 783, 784, 785, MP001, MP006, MP063, MP068, MP086, MP087, MP088, MP089, MP090, MP110, MP123, MP124, MP126, MP142, MP156, MP167, MP170, MP188, MP191, MP197, MP198, MP199, MP200, MP207, MP215, MP217, MP220, MP224, MP229, MP235, MP236, MP242, TP009, TP016, TP031, TP036, TP064, TP085, TP102, TP104, TP153, TP154, TP156, TP158, TP160, TP165, TP166, TP169, TP189, TP195, TP201, TP234, TP237, TP239, TP270, WP014, WP015, WP016, WP019, WP040, WP041, WP060, WP062, WP069, WP073, WP089, WP096, WP112, WP118, WP125, WP126, WP156, WP181, WP191, WP200, WP202, WP233, WP234, WP241, WP271, RP001, RP008, RP024, RP026, RP030, RP031, RP036, RP039, RP060, RP096, RP102, RP106, RP111, RP114, RP124, RP167, RP170, RP188, RP193, RP200, RP213, RP215, RP227, RP292
- Risk management** 6, 8, 48, 84, 102, 110, 173, 181, 184, 185, 188, 189, 193, 245, 282, 283, 285, 359, 396, 398, 403, 423, 425, 427, 428, 478, 480, 481, 483, 501, 581, 586, 662, 690, 714, 731, 733, 742, 749, 751, 772, 774, 783, 786, MP083, MP084, MP167, MP205, MP215, MP221, MP223, MP224, MP242, TP158, TP159, TP234, TP253, WP001, WP016, WP061, WP064, WP078, WP079, WP087, WP092, WP095, WP126, WP127, WP244, WP252, RP086, RP118, RP193
- Roadway** 554, WP053, RP023, RP025, RP125, RP184
- Sediment** 24, 31, 69, 77, 78, 82, 85, 86, 87, 88, 90, 91, 106, 107, 160, 161, 185, 186, 188, 189, 190, 191, 192, 193, 194, 203, 211, 217, 228, 229, 255, 258, 260, 261, 264, 297, 326, 341, 359, 361, 362, 397, 407, 423, 429, 433, 446, 447, 450, 451, 481, 485, 542, 601, 605, 616, 618, 619, 646, 647, 649, 652, 657, 659, 669, 671, 672, 693, 694, 695, 696, 698, 699, 700, 755, 756, 757, 758, 761, MP008, MP022, MP027, MP028, MP030, MP048, MP079, MP087, MP095, MP101, MP102, MP107, MP110, MP114, MP117, MP128, MP130, MP131, MP134, MP135, MP177, MP179, MP204, MP210, MP216, MP224, MP225, MP231, MP234, MP237, MP238, MP239, MP240, MP241, TP026, TP035, TP036, TP046, TP050, TP054, TP055, TP064, TP071, TP094, TP095, TP145, TP150, TP151, TP157, TP159, TP167, TP207, TP208, TP213, TP264, WP005, WP009, WP019, WP020, WP030, WP039, WP041, WP052, WP067, WP069, WP085, WP094, WP119, WP147, WP173, WP190, WP198, WP199, WP230, WP241, WP242, WP243, WP244, WP245, WP246, WP247, WP248, WP250, RP017, RP023, RP035, RP036, RP040, RP047, RP048, RP059, RP061, RP062, RP063, RP066, RP074, RP100, RP105, RP110, RP123, RP140, RP155, RP162, RP163, RP181, RP186, RP213, RP242, RP264, RP266, RP267
- Soil** 56, 84, 89, 127, 183, 186, 248, 250, 259, 291, 292, 293, 294, 303, 320, 322, 324, 327, 352, 359, 386, 387, 388, 483, 546, 553, 574, 575, 576, 577, 578, 579, 580, 600, 604, 631, 640, 646, 648, 658, 703, 706, 735, 746, 766, 774, 775, MP026, MP087, MP096, MP097, MP098, MP105, MP115, MP132, MP171, MP196, TP067, TP072, TP099, TP100, TP101, TP104, TP145, TP161, TP163, TP173, TP174, TP182, TP184, TP186, TP189, TP199, TP267, WP009, WP022, WP023, WP123, WP124, WP125, WP154, WP177, WP190, WP198, WP233, WP236, WP237, WP238, WP247, WP249, WP262, WP263, WP271, WP280, WP284, RP001, RP002, RP022, RP059, RP062, RP063, RP121, RP122, RP124, RP128, RP129
- Sorption** 24, 60, 91, 140, 387, 507, 655, 658, 659, 660, 738, 755, 758, 760, 761, 762, MP002, MP186, TP011, TP012, TP088, TP098, TP100, TP104, TP122, TP169, TP186, TP238, TP267, WP029, RP126, RP223
- Spatial** 67, 103, 156, 167, 169, 255, 256, 274, 443, 475, 671, 776, MP023, MP084, MP167, MP178, MP206, TP061, TP077, TP147, TP216, WP014, WP081, WP179, WP266, WP283, RP051, RP116, RP119, RP254
- Speciation** 31, 89, 230, 233, 268, 409, 411, 573, 597, 602, 603, 700, 702, 706, 774, 775, MP026, MP027, MP121, TP243, WP029, WP129, WP131, WP193, WP196, WP205, WP211, WP233, RP031, RP070, RP124, RP126, RP255, RP293
- Statistics** 106, 225, 293, 329, 536, 583, 594, 601, 647, 661, 664, 677, 682, 683, 693, 718, 721, 770, MP003, MP065, MP106, MP216, MP225, MP227, WP073, WP074, WP078, RP101, RP106, RP156, RP262
- Stormwater** 153, 552, 554, 650, 723, 727, 728, 729, 730, MP111, TP157, TP159, RP011, RP012, RP013, RP016, RP017, RP018, RP019, RP021, RP022, RP023, RP024, RP026, RP027, RP125, RP143, RP198, RP236, RP269
- Surface water** 21, 36, 38, 57, 84, 105, 153, 158, 162, 230, 246, 255, 261, 264, 274, 275, 276, 281, 299, 305, 345, 432, 436, 437, 449, 474, 479, 484, 535, 555, 612, 639, 654, 656, 673, 723, 725, 726, 756, 781, MP003, MP011, MP017, MP038, MP056, MP057, MP088, MP106, MP120, MP135, MP136, MP139, MP145, MP184, TP003, TP005, TP006, TP011, TP016, TP028, TP040, TP041, TP093, TP096, TP175, TP177, TP202, TP215, TP232, TP246, TP260, TP261, TP265, TP270, WP005, WP014, WP031, WP038, WP083, WP088, WP120, WP137, WP196, WP232, RP032, RP036, RP041, RP045, RP049, RP052, RP103, RP171, RP183, RP197, RP233, RP275
- Sustainability** 3, 72, 401, 404, 423, 429, 478, 582, 585, 586, 587, 588, 686, 687, 688, 690, 772, 774, MP093, MP109, MP226, TP002, TP181, WP050, WP123, RP024, RP085, RP113, RP115, RP117, RP283, RP284
- Systems analysis** 6, 225, 307, 342, 378, 423, 465, 487, 488, 491, 493, 679, 680, 681, 772, MP085, MP143, MP228, MP233, WP090, WP092, WP208, RP087, RP282, RP284
- Toxicity** 2, 19, 23, 40, 56, 88, 119, 132, 136, 139, 140, 146, 218, 219, 229, 235, 241, 249, 253, 287, 293, 294, 315, 317, 318, 319, 321, 322, 323, 324, 326, 333, 336, 338, 342, 352, 364, 378, 380, 384, 385, 386, 389, 390, 392.5, 394, 400, 402, 412, 414, 416, 446, 451, 452, 454, 459, 462, 498, 508, 510, 511, 520, 522, 532, 565, 572, 581, 583, 585, 588, 602, 616, 617, 620, 621, 626, 629, 685, 687, 688, 691, 696, 702, 703, 707, 709, 720, 730, 731, 732, 738, 757, 765, 767, MP023, MP060, MP061, MP076, MP089, MP090, MP098, MP108, MP125, MP153, MP158, MP160, MP197, MP207, MP238, MP240, MP242, TP014, TP032, TP053, TP071, TP072, TP103, TP104, TP107, TP108, TP111, TP113, TP121, TP124, TP126, TP136, TP137, TP140, TP150, TP151, TP152, TP153, TP155, TP163, TP165, TP173, TP176, TP177, TP182, TP184, TP189, TP219, TP220, TP221, TP251, TP268, WP030, WP034, WP035, WP047, WP049, WP057, WP068, WP101, WP103, WP115, WP131, WP140, WP150, WP159, WP164, WP181, WP187, WP197, WP210, WP213, WP230, WP232, WP237, WP239, WP242, WP253, WP254, WP259, WP263, WP265, WP268, WP273, WP280, WP282, WP283, WP284, WP285, WP286, RP006, RP007, RP023, RP031, RP033, RP036, RP038, RP055, RP057, RP058, RP062, RP069, RP080, RP083, RP101, RP110, RP127, RP144, RP148, RP151, RP160, RP163, RP166, RP169, RP178, RP205, RP208, RP235, RP249, RP278, RP291, RP293
- Toxicokinetics** 207, 208, 384, 492, 623, MP159, TP065, TP207, TP212, WP184
- Uncertainty** 209, 312, 478, 623, 642, 682, 714, 715, MP042, MP186, MP216, MP220, MP235, TP081, TP231, TP236, WP091, WP127, RP039, RP086, RP096, RP118, RP119, RP266
- Urban** 13, 49, 71, 101, 170, 180, 256, 359, 439, 458, 537, 554, 555, 556, 557, 600, 604, 643, 723, 724, 725, 728, 729, MP014, MP017, MP050, MP055, MP087, MP117, MP154, MP229, MP234, TP058, TP094, TP147, TP148, TP157, WP015, WP023, WP179, WP180, RP002, RP008, RP011, RP014, RP015, RP020, RP021, RP022, RP024, RP026, RP027, RP051, RP052, RP125, RP228, RP293
- Waste water** 20, 21, 99, 153, 154, 159, 221, 250, 254, 261, 298, 299, 301, 311, 357, 358, 436, 461, 472, 515, 527, 567, 568, 569, 571, 589, 592, 594, 595, 596, 657, 694, 718, MP005, MP009, MP024, MP081, MP137, MP144, MP168, MP192, MP218, TP002, TP003, TP055, TP060, TP062, TP068, TP070, TP073, TP094, TP118, TP130, TP169, TP215, TP241, TP244, TP250, TP251, TP261, TP263, TP271, WP004, WP015, WP035, WP042, WP044, WP074, WP155, RP012, RP013, RP015, RP032, RP040, RP042, RP147, RP162, RP184, RP233
- Water quality** 38, 55, 57, 68, 111, 112, 113, 115, 135, 151, 157, 161, 168, 217, 226, 247, 272, 274, 278, 304, 335, 341, 344, 345, 346, 347, 348, 351, 360, 391, 405, 421, 422, 444, 473, 484, 535, 557, 589, 590, 595, 596, 661, 662, 663, 668, 670, 697, 699, 721, 723, 726, 728, 730, MP037, MP038, MP039, MP041, MP076, MP146, MP183, MP184, MP222, MP223, TP001, TP002, TP004, TP005, TP006, TP007, TP012, TP015, TP016, TP017, TP028, TP036, TP037, TP048, TP078, TP079, TP128, TP204, TP223, TP243, TP255, TP260, WP001, WP011, WP031, WP032, WP099, WP100, WP116, WP118, WP130, WP194, WP210, WP221, WP224, WP227, RP012, RP016, RP017, RP019, RP020, RP021, RP025, RP028, RP036, RP039, RP041, RP045, RP052, RP071, RP085, RP088, RP104, RP142, RP185, RP196, RP257, RP267
- Weight of evidence** 28, 96, 121, 123, 150, 209, 217, 296, 328, 332, 447, 527, 643, 743, MP162, MP172, MP185, MP197, MP204, TP082, TP085, WP059, WP109, WP118, RP137, RP290
- Wetlands** 37, 247, 248, 289, 358, 392.5, 420, 422, 425, 545, 577, 612, 744, 751, MP027, MP036, MP043, MP105, MP133, MP189, TP046, TP224, WP044, WP053, WP061, WP267, RP089, RP130, RP134, RP145, RP147, RP164, RP185, RP202

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## Administrative Offices:

### Pensacola Office

229 S Baylen St., 2<sup>nd</sup> Floor

Pensacola, FL 32502

USA

T +1 850 469 1500

F +1 850 469 9778

E [setac@setac.org](mailto:setac@setac.org)

### Brussels Office

Av. de la Toison d'Or 67

B-1060 Brussels

Belgium

T +32 2 772 72 81

F +32 2 770 53 86

E [setaceu@setac.org](mailto:setaceu@setac.org)

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